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THE BIOPHARMACEUTICS AND PHARMACOKINETICS OF SOME SUBSTITUTED SUCCINIMIDES IN MALE WISTAR RATS

by

© SHABIR ZAHOOR MASIH

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF DOCTOR OF PHILOSOPHY

IN

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EDMONTON, ALBERTA

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled, "The Biopharmaceutics and Pharmacokinetics of Some Substituted Succinimides in Male Wistar Rats," submitted by Shabir Zahoor Masih in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Biopharmaceutics.

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Supervisor

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External Examiner

Date July 5, 1974

This Thesis is Dedicated to

MY MOTHER, Ashrita Z. Masih, for all the sufferings she went through in pushing me into intellectual pursuits against my wishes in my early adulthood;

MY WIFE, Manjula Masih, for her patience, understanding, and whole-hearted support of my endeavours;

MY SONS, Salim, Sylvanus, Anil and Aaron for foregoing, without resentment, their legitimate needs and pleasures of early childhood to see me through this education;

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TO THE CAUSE OF CHRISTIAN MISSIONS, which has filled my heart with a new song and given a meaning and purpose for my life.

ABSTRACT

The kinetics of the absorption, distribution and elimination of ethosuximide, methsuximide and phensuximide have been examined in male Wistar rats following intravenous or oral administration of each drug. Ethosuximide, methsuximide and phensuximide were determined by gas-liquid chromotography in blood, urine, or tissue homogenates using a modification of a published technique which gave better sensitivity and reproducibility. Various pharmacokinetic parameters were determined from a study of the blood level vs. time profiles and the urinary excretion rates of the drugs. The pharmacokinetic rate constants, and other derived parameters, were obtained from blood level or urinary excretion data by using a digital computer program, "NONLIN", to fit a number of pharmacokinetic models using an iterative, "least square" fitting procedure.

The relationship between the dose administered intravenously and the kinetic parameters of ethosuximide was examined following intravenous administration of the drug. The kinetics of ethosuximide following multiple oral dosing were also examined and some of the information obtained has been used to predict blood concentrations in man from previously published data.

The urinary excretion of ethosuximide has been examined in male Wistar rats pretreated with ammonium chloride, sodium bicarbonate and probenicid. None of these pretreatments was found to alter the amount of an administered dose excreted unchanged in the urine when compared with the amounts found in the urine of animals not pretreated. This suggests that, since the drug was found to be negligibly bound to plasma proteins, the long half-life observed for ethosuximide in rats, in com-

parison with methsuximide or phensuximide, is due to the slow rate of metabolism of the drug and extensive passive tubular reabsorption.

The influence of some pharmaceutical adjuvants and antacids on the systemic availability of ethosuximide following oral administration to rats has been studied. Zarontin^{R} syrup, Zarontin^{R} capsules and ethosuximide, in aqueous solution, were all equally available. Most of the adjuvants examined increased the time taken to achieve peak plasma levels suggesting a reduced rate of absorption. Small reductions in availability were also noted. Soluble calcium salts and antacids containing either calcium or magnesium significantly reduced the availability of ethosuximide.

The significance of these studies, in terms of the design of dosage forms and the clinical implications of the various dosage regimens for ethosuximide, have been discussed in the light of the pharmacokinetic findings.

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CHAPTER I

INTRODUCTION

Pharmacokinetics has been defined as 'the mathematical description of drug quantity and activity changes within the body' (Portmann, 1970). Thus, pharmacokinetics is really an attempt to describe the processes of absorption, distribution and clearance, undergone by a drug in the body, in mathematical terms. When this has been accomplished for a particular drug, it should then be possible to forecast what will happen to a particular drug in an animal species, and to predict the amount of drug which may be found in body fluids or tissues at any given time. Thus, a knowledge of the pharmacokinetic parameters of a drug has obvious importance in the use of that drug to treat disease, and is especially useful in devising dosage schedules to attain, and maintain, therapeutic concentrations of the drug in the body. In addition to this, pharmacokinetics can be used to avoid ineffective therapy, due to the drug being present in ineffective concentrations, or to avoid toxicity, due to the accumulation of the drug in the body.

Although pharmacokinetics has only recently been introduced into the curriculum of most schools of pharmacy, it is not a new branch of science. In the 1920's, Widmark (1919) and Widmark and Tandberg (1924) applied the principles of kinetic analysis to drug elimination, and to the changes in drug levels which take place during multiple dosing. Other pioneers in this field were Gehlen (1933), Dominguez (1935) and Beccari (1938). Since 1950, pharmacokinetics has expanded rapidly and is currently one of the most important branches of pharmaceutics.

Most pharmacokinetic studies use models which are based on the physiological concepts of absorption, distribution and clearance. Despite the fact that these processes may be very complex and composed of a large number of individual processes, most of the overall processes

can be described by simple first order kinetics. This results in relatively simple mathematical descriptions of the kinetic processes involved, and makes possible the use of greatly simplified mathematical models to describe the pharmacokinetics of the drug in the body. The use of models in pharmacokinetics has been well reviewed by Teorell (1937a,b), Dost (1953) and Nelson (1961a). In pharmacokinetics, the models usually consist of a series of compartments. A compartment may be defined as a kinetically distinguishable pool in terms of the drug concentration-time profile. The drug distributes between these various compartments. Clearance, that is the metabolism and excretion of the drug, may take place from one or more of these compartments. It is difficult to define in material terms what constitutes a 'compartment' in pharmacokinetics. It may be considered to be a group of different tissues which have certain characteristics in common, such as high or low blood flow rates or the solvent characteristics of the tissue. Eger (1963) divided the tissues of the body up into groups using these criteria. His first group contained tissues which were profusely supplied with blood vessels and included the heart, brain, spinal cord, hepato-portal system, kidney and endocrine glands. This group accounted for approximately 9% of the body weight. A second group, the muscle group, was made up of skin and muscle and formed about 50% of the total body weight. Adipose tissue and bone marrow formed a third group, the fat group, comprising about 19% of the total body weight. The last group, the vessel-poor group, contained bone, ligaments, cartilage, tendons, teeth and hair and made up about 22% of the total body weight. Price (1963) considered blood flow rates to be the most important factor in determining the availability of a drug to a specific tissue. He cited his studies with thiopental (Price, 1960) in support of his

suggestions. However, the relationship between drug uptake and tissue blood flow is often not exact because the partition coefficient between plasma and different body tissues varies. The partition coefficient is determined by many factors, including the lipid solubility of the drug, its degree of ionization, its molecular size and presence or absence of binding to plasma proteins. However, the fact that approximately 70% of the cardiac output supplies about 7% of the total body tissue, emphasizes the importance of blood flow rates in determining the division of the body tissues into compartments. Reigelman, Loo and Rowland (1968), considering the concept of a two compartment model (this will be discussed in detail later), suggested that the central compartment consisted of the blood, highly perfused tissues, such as the brain, and some extravascular fluids. The liver also contributed to this compartment and, to a lesser extent, the kidneys and the gastrointestinal tract. Fat and poorly perfused tissues made up the tissue or peripheral compartment. It is apparent from the above review that it is the physicochemical properties of the drug which will determine the tissues which form the various compartments and that this will be influenced by the blood flow to the various tissues. Whatever the composition of these compartments, or kinetically distinguishable pools, in a model they are connected by irreversible or reversible pathways which represent the movement of drug into, between and out of the various compartments.

The use of compartmental models to explain the behaviour of a drug in the body is fraught with difficulties and pitfalls. Berlin et al. (1968) and Berman (1969) have outlined the essential steps which must be followed in the construction and application of compartmental models. Usually the simplest possible model is used first. It is fitted to the data, often using an iterative process, and the sums of the squares of

the deviations of the experimental points from the calculated points are used to assess how closely the experimental data approximates to the calculated data points. Other more complex models are then fitted to the data, until there is no statistically significant reduction in the variance of the experimental data from the calculated data. A degree of empiricism or pragmatism is sometimes necessary to avoid unnecessarily complicated models and to ensure that a good fit is obtained with physiologically meaningful parameters. The model is then tested experimentally in as many situations as possible. If it still provides a good explanation of the data obtained, it is acceptable; but in many instances the new information obtained results in revision of the proposed model to accomodate the new data. Generally speaking, each revision will make the model more accurate in predicting other data.

Several different models may be used for a particular set of data. Often the governing reason for selecting a particular model is the information it is hoped to predict. This aspect has been discussed by Berman (1969). Each model is limited by the fact that it is only possible, practically, to sample a small number of compartments. This, coupled with the fact that compartments are difficult to define, even for a particular drug, limits the uniqueness of any model system. If one considers that one usually only samples one compartment, such as plasma, then it obviously requires considerable intuition or imagination to apply a three or four compartmental model to the kinetics of a drug! Thus, it has to be recognized that each model represents a gross oversimplification of the real system and that each pharmacokinetic parameter is in reality the sum or the combination of a large number of different rate processes. Because of this, each rate constant determined for a given model only has value in the context of that particular model and

cannot be applied universally. With this preamble it is now possible to go on to consider some of the various pharmacokinetic models which have been shown to have utility.

The concepts of pharmacokinetics and compartmental analysis have been systematically presented in a number of publications (Gibaldi, 1971; Notari, 1971; Solomon, 1960; Swarbrick, 1970; Swarbrick, 1973; Wagner, 1971a).

The simplest pharmacokinetic approach considers the animal or organism to consist of one compartment. This is shown schematically in Figure 1a. According to this scheme, the body is considered to be a single unit separated from the environment by a membrane which is permeable to drugs. If a drug is given intravenously, it is assumed that the drug is instantaneously and homogenously distributed throughout this single compartment. It is also assumed that the drug is eliminated from this compartment by a process of metabolism and excretion which can be described by first order kinetics. The amount of drug in the body or compartment can then be described by Equation (1)

$$C_c = \frac{D}{V} \cdot e^{-Kt} \quad \ldots\ldots\ldots\ldots (1)$$

where 'C_c' is the concentration of the drug in the single compartment at time 't', 'D' is the dose administered and 'V' is the apparent volume of distribution, which may be defined as the volume of body fluid in which the drug appears to be dissolved, assuming a uniform distribution, and 'K' is the rate constant for the elimination of the drug by both metabolism and excretion.

A term which has significance is

$$\frac{D}{V} = C_o \quad \ldots\ldots\ldots\ldots\ldots\ldots (2)$$

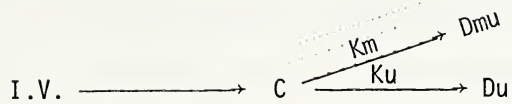

Figure 1a. A scheme showing a one compartment pharmacokinetic model for a drug following intravenous administration.

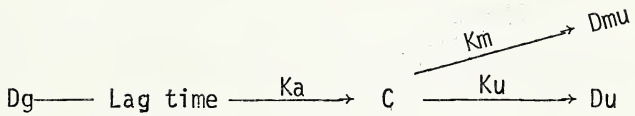

Figure 1b. A scheme showing a one ("two") compartment pharmacokinetic model for a drug following oral administration.

This will give 'C_o' the fictional initial concentration of the drug in the compartment.

Also

$$K = K_u + K_m \quad \ldots\ldots\ldots\ldots\ldots (3)$$

where 'K_u' is the rate constant for the urinary excretion of the unchanged drug and 'K_m' is the rate constant for the removal of the drug by metabolism.

A single compartment model of this type has been described, in depth, by Teorell (1937b) and it was used by Widmark (1919) and Widmark and Tandberg (1924) to examine the blood concentration - time profile of some narcotic substances. The model gives several interesting results. For example when the drug is extensively bound to plasma proteins, or red blood cells, since this is the part of the compartment usually sampled, the apparent volume of distribution will be less than the actual plasma volume. In contrast, a high value for the volume of distribution would be an indication of the localization and concentration of the drug in body tissues other than blood. Thus it is important to have a good knowledge of the degree of plasma protein binding and tissue localization a drug undergoes in order to fully understand the kinetics of a drug. If this information were not available misleading conclusions regarding the therapeutic performance of the drug could be made. A good example of the value of this data was produced by Sutherland (1962) who examined and compared blood and tissue levels of spiramycin and erythromycin. An excellent discussion of the effects of plasma protein binding on drug distribution has been presented by Martin (1965a). Also, Martin (1965b) showed that plasma protein binding not only influences the distribution of drugs in the body but also affects the elimination of drugs from the body. This is because drug bound to protein is not available for

metabolism nor can it be excreted by filtration through the renal tubules. Thus the biological half-life of a drug which is extensively protein bound is often quite long. The fact that plasma protein binding affects the pharmacokinetic parameters for a drug has been shown by several authors (Anton, 1961; Brodie and Hogben, 1957; Weiner et al., 1950; Wiseman and Nelson, 1964).

When the body is considered as a single compartment, and the drug is given other than intravenously, for example orally, the pharmacokinetic model needs to be modified as may be seen in Figure 1b. The only difference from the single compartment model for intravenous dosing shown in Figure 1a is the introduction of a term D_g. This represents the amount of drug given orally, which, after a suitable lag time, undergoes disintegration and dissolution and absorption into the single compartment. The rate constant for absorption is termed K_a. The compartment drug level-time profile is then described by equation (4) (assuming all the drug is absorbed).

$$C_c = \frac{D}{V} \cdot \frac{K_a}{K_a - K} \left(e^{-Kt} - e^{-K_a t}\right) \quad . \quad . \quad . \quad . \quad . \quad (4)$$

Examination of this equation shows that the concentration of drug in the compartment will increase until it reaches a maximum which will then decline. This is because the rate of drug elimination up to the peak level is less than the rate of absorption. After the maximum the reverse is true. The time of occurrence of the maximum drug level (t_{max}) is independent of dose depending only on K_a and K; t_{max} can be obtained from equation (5).

$$t_{max} = \frac{1}{K_a - K} \cdot \ln \frac{K_a}{K} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (5)$$

Note, however, that the magnitude of the peak compartmental concentration is a function of the size of the dose administered according to equation (7).

$$C_{max} = \frac{D}{V} \cdot \left(\frac{K_a}{K}\right)^{\frac{K}{K_a-K}} \quad \ldots\ldots\ldots (6)$$

When the absorption of the drug from its site of administration is incomplete, equation (4) has to be modified to give equation (7).

$$C_c = \frac{FD}{V} \cdot \frac{K_a}{K_a-K} \cdot \left(e^{-Kt} - e^{-K_a t}\right) \quad \ldots (7)$$

where F is the fraction of the dose absorbed. In equation (7), when K_a is at least two to three times greater than K, which is often the case, the contribution of the term '$-e^{-K_a t}$' becomes negligible. Therefore, the slope of the terminal portion of the straight line which results from a semilogarithmic plot of the compartment concentration-time data yields the elimination rate constant 'K'.

Since the shape of the compartment concentration-time curve generated by equation (7) depends on the difference between the products of a constant and the exponential terms e^{-Kt} and $e^{-K_a t}$, the absorption rate constant, K_a, may be determined by subtracting the compartment concentration from the ascending portion of the curve from the estimated concentrations which result when the descending portion of the curve is extrapolated back to the ordinate. This technique is referred to as 'feathering'.

The estimation of 'F', the fraction of dose absorbed per volume of distribution, in equation (7) can thus be accomplished by comparing the areas under the compartment concentration-time curves obtained after the administration of the same amount of drug by the intravenous and other

routes. This is best done in the same animal if at all possible. This procedure offers an excellent way of studying the influence of varying the physicochemical properties and formulation of the drug on absorption. By determining K_a, F, t_{max} and the area under the curve (AUC) for various polymorphic forms of the drug, for various adjuvants and diluents and for various dosage forms, it is possible to compare formulations by a number of different routes. Studies such as this are important since variations in manufacturing processes may influence the rate and extent of absorption of a drug from its dosage form (Goldstein, 1968; D.I.B., 1969). Martin et al. (1968) have noted differences in the availability of preparations of diphenylhydantoin, sulfisoxazole and chloramphenicol produced by different manufacturers. Many more examples are known but one of the most recent and significant refers to the availability of digoxin from the products of various manufacturers (Wagner et al., 1973a).

Methods of estimating the bioavailability of drugs and the problems associated with these determinations have been discussed by Beckett and Tucker (1967), Dittert and Disanto (1973) and Wagner (1966). The methods are essentially those described above but the authors discuss the practical and computational difficulties which have to be resolved to obtain meaningful data.

In most instances, however, drugs are not given once only, but according to a schedule. In the majority of cases the plasma level of a drug relates directly to its therapeutic or toxic effects (Davies and Pritchard, 1973). Thus the plasma level-time profile can be used to avoid toxic concentrations of drug in plasma (Nelson and Kruger-Thiemer, 1964; O'Reilly, 1972; Wagner and Metzler, 1969). As has been pointed out by some of these authors, a dosing schedule need not be empirical

but should be based on a knowledge of the kinetics of the drug being used in that particular animal or patient.

If the same dose 'D' of a drug is administered repeatedly at a fixed interval of time 'J'. The amount of drug present in the compartment, in this case the term plasma will be used, after the nth administration is given by equation (8).

$$C_n = \frac{FD}{V} \cdot \frac{K_a}{K_a - K} \left[\left(e^{-Kt} \cdot \frac{1 - e^{-nKJ}}{1 - e^{-KJ}} \right) - \left(e^{-K_a t} \cdot \frac{1 - e^{-nK_aJ}}{1 - e^{-K_aJ}} \right) \right] \quad . \quad . \quad (8)$$

where C_n is the plasma concentration of the drug after the nth dosing.

The degree of drug accumulation after multiple dosing may be ascertained from the drug accumulation ratio, 'R'. This may be defined as the ratio of the average amount of drug in the body, at the time a plateau concentration is reached, to the amount of drug absorbed following a single dose according to equation (9).

$$R = \frac{\bar{C}_\infty V}{FD} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (9)$$

where $\bar{C}_\infty$ is the average concentration of the drug in the body after administration of 'n' doses.

Since

$$\bar{C}_\infty = \frac{FD}{V K J} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (10)$$

substituting for $\bar{C}_\infty$ in equation (9) we obtain

$$R = \frac{1}{K J} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (11)$$

This relationship indicates that the drug-accumulation ratio is dependent on the overall elimination rate constant, K, (where $K = 0.693/t_{1/2}$) and the dosing interval, J. In terms of biological half life ($t_{1/2}$), the

accumulation ratio, R, may be expressed as:

$$R = \frac{1.44\ t_{1/2}}{J} \quad . \ . \ . \ . \ . \ . \ . \ . \ . \ (12)$$

Thus the dosing interval should not be less than 1.44 times the biological half life of the drug to prevent toxic manifestations from drug accumulation. Holcenberg (1970), as a physician, emphasized the usefulness of a knowledge of the biological half life of the drug for deciding proper dosage scheduling in the therapeutic management of the patient. An entire symposium was sponsored by the Hoechst Laboratories in 1970 to emphasize this aspect of pharmacokinetics, and the papers presented at the symposium have been published in the form of a book (Dengler 1970).

Although the concept of the one compartment pharmacokinetic model has been found useful in characterizing the dynamics of drug absorption, distribution, metabolism and excretion, Dominquez (1950) pointed out that one may arrive at a better understanding of the pharmacokinetic behaviour of a drug in the body, if the body is considered as a system divided into more than one interconnected compartments into which the drug distributes itself. Rescigno and Segre (1966) and Riggs (1963) have presented detailed accounts of the mathematical basis of such compartmental analyses. These authors have presented various mathematical models which consider the body to consist of one or more compartments connected together in diverse manners. One or other of these models should be quite adequate to describe the plasma level-time profile of most drugs.

The kinetic behaviour of a multicompartment system can be described mathematically by a general system of linear first-order differential equations of the type:

$$\frac{dD_i}{dt} = \sum_{j=0}^{n} K_{ij} D_j - \sum_{j=1}^{n} K_{ij} D_i \quad . \quad . \quad . \quad . \quad (13)$$

where D_i and D_j represent the actual amount of drug in the i^{th} and j^{th} compartment respectively, and K_{ij} stands for the rate constant for the transfer of drug from i^{th} to the j^{th} compartment. Reigelman et al. (1968) tested experimentally the validity of one and two compartment pharmacokinetic models by comparing the results of the pharmacokinetic analysis of experimental blood level data, using a one and two compartment system. They showed that for some drugs, whose pharmacokinetic behaviour cannot be explained adequately with a one compartment pharmacokinetic model, a two compartment pharmacokinetic model seemed to offer results which were consistent with the experimental data. A two compartment model used in this investigation is shown schematically in Figures 2a and 2b.

In Figure 2a, C_c is the concentration of the drug in the central compartment; C, into which the drug is directly administered by an intravenous administration. The central compartment C, includes the blood, other body fluids, and the organs and tissues highly perfused with blood; T is the peripheral compartment into which the drug from the central compartment, C, is distributed; Kct is the first order rate constant for the transfer of drug from the central compartment C into the peripheral compartment T; Ktc, is the first order rate constant for the return of the drug from the peripheral compartment T into the central compartment C, for metabolism and excretion. It is assumed that all metabolism and excretion occurs from the central compartment; Ku is the first order rate constant for excretion of the unchanged drug into urine; and Km is the first order rate constant for the metabolism of the drug. The overall elimination rate constant, K, which includes the rate constants for

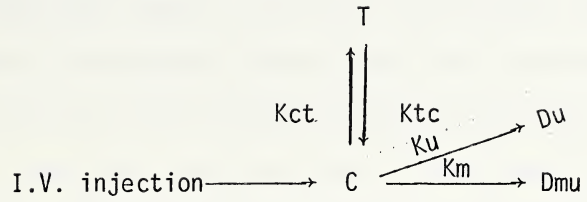

Figure 2a. A scheme showing a two compartment pharmacokinetic model for a drug following intravenous administration.

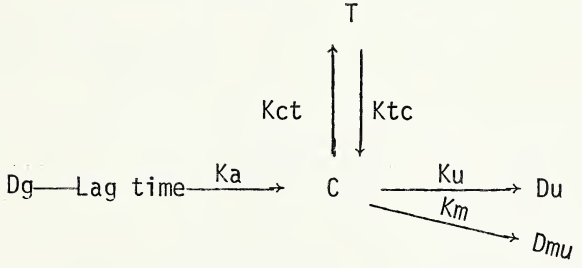

Figure 2b. A scheme showing a two compartment pharmacokinetic model for a drug following oral administration.

metabolism and excretion, differs from the overall rate constant K of a one compartment model in that the K of the one compartment model corresponds to the hybrid rate constant 'β' of the two compartment model. Du and Dmu have been defined previously for a one compartment model. Based on the two compartment model scheme shown in Figure 2a, the blood level-time curve can be described by equation (14).

$$C_c = A.e^{-\alpha t} + B.e^{-\beta t} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (14)$$

where at t = 0, the initial plasma concentration $C_o = A + B$, and α and β are hybrid rate constants each influenced by all the individual rate processes. The knowledge of the concentration of the drug in the central compartment C as a function of time permits the estimation of other rate constants and related pharmacokinetic parameter according to equations 15 through 25.

$$Kct = \frac{A.B.\ (\beta - \alpha)^2}{(A+B)\ (A\beta+B\alpha)} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (15)$$

$$Ktc = \frac{A\beta + B\alpha}{A + B} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (16)$$

$$K = Ku + Km = \frac{\alpha\beta(A + B)}{A\beta + B\alpha} = \frac{\alpha\beta}{Ktc} \quad . \quad . \quad . \quad (17)$$

$$\alpha = 1/2\ (Kct + K + Ktc) + \sqrt{(Kct + K + Ktc)^2 - 4Ktc.K} \quad . \quad (18)$$

$$\beta = 1/2\ (Kct + K + Ktc) - \sqrt{(Kct + K + Ktc)^2 - 4Ktc.K} \quad . \quad (19)$$

$$t_{1/2} = \frac{0.693}{\beta} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (20)$$

$$V_c = \frac{D}{A + B} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (21)$$

where V_c is the volume of distribution for the drug in the central compartment.

$$V_t = \frac{V_c . Kct}{Ktc} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (22)$$

where V_t is the volume of distribution of the drug in the peripheral compartment.

$$D_t = \frac{Kct.D}{(\alpha-\beta)} \left(e^{-\alpha t} + e^{-\beta t}\right) \quad . \quad . \quad . \quad . \quad . \quad (23)$$

where D_t is the amount of drug in the peripheral compartment.

$$Du = D.Ku \left[\frac{1}{K} - \left(\frac{A.e^{-\alpha t}}{\alpha} + \frac{B.e^{-\beta t}}{\beta}\right)\right] \quad . \quad . \quad (24)$$

where Du is the amount of unchanged drug excreted in the urine.

$$AUC = \frac{A}{\alpha} + \frac{B}{\beta} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (25)$$

When the disappearance of the drug from the central compartment as a function of time, following an intravenous dosing, shows a biphasic behaviour (see Figure 16), the analysis of the blood level data requires a minimum of two compartments in a model. However, if the first phase showing the rapid decline in plasma concentration is not seen because of very little sampling during the initial phase, an erroneous estimation of the drug distribution volume V is obtained, and one is tempted to use the terminal phase of the drug-disappearance curve for the determination of biological half life according to a one compartment model. Riegelman

(1970) has cautioned against such mistakes in the analysis of plasma level data. He strongly advises the experimenter to take a sufficient number of blood samples immediately following the intravenous administration at short intervals so as not to miss the fast distribution phase.

In the two compartment model in which an absorption step is included, Dg, the amount of drug in the absorption compartment (this is most often the gastrointestinal tract) is absorbed into the central compartment C as shown in Figure 2b. Under these circumstances the concentration of drug in the central compartment at time 't' is given by the equation

$$C_c = A.e^{-\alpha t} + B.e^{-\beta t} - C.e^{-Kat} \quad . \quad . \quad . \quad . \quad . \quad (26)$$

where $C.e^{-Kat}$ represents the amount of drug remaining in the gut at time 't'.

The process of drug absorption, distribution, metabolism and excretion can also be studied by observing the amount of drug excreted unchanged in the urine after various time intervals. This method has some advantages over methods involving studies of blood levels, particularly in cases in which the amount of drug found in blood is below the detection limits of known analytical methods. Urine usually contains relatively large amounts of unchanged drug and is available in greater volumes than blood. Studies of urinary excretion also have the advantage that they do not require a knowledge of the apparent volume of distribution. The rate of excretion of unchanged drug in the urine can be described by equation (27).

$$\frac{d\,Du}{dt} = K_u\, D.e^{-Kt} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (27)$$

where $\frac{d\,Du}{dt}$ is the excretion rate at time 't'. Integration gives equation (28).

$$Du = \frac{K_u}{K} \quad . \quad D \quad . \quad \left(1 - e^{-Kt}\right) \quad . \quad . \quad . \quad . \quad . \quad (28)$$

The above equation assumes that the drug is given intravenously. If the drug is given orally, equations 29 - 31 may be used to analyse data obtained in a urinary excretion study.

$$\frac{dDu}{dt} = F \,.\, D \,.\, \frac{K_u \,.\, K_a}{K_a - K} \left(e^{-Kt} - e^{-K_a t}\right) \quad . \quad (29)$$

$$Du = \frac{F \,.\, D \,.\, Ku}{K.(K_a - K)} \quad . \quad \left[K_a \left(1 - e^{-Kt}\right) - K \left(1 - e^{-K_a t}\right)\right] \quad . \quad (30)$$

$$Du_\infty = F \,.\, D \,.\, \frac{K_u}{K} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (31)$$

where Du_∞ is the total amount of drug excreted in the urine after infinite time. This is usually considered to be equivalent to not less than six half lives ($t_{1/2}$) of the drug. In any urinary excretion study simultaneous determinations of plasma levels of drug have great value in determining the kinetic constants of the drug for a particular model. In addition to this it is often wise to study the urinary excretion of both the unchanged drug and its metabolites. The reason for this may be seen in the work of Barr (1969). Barr showed that in the case of salicylamide, the drug appeared to be very poorly absorbed

when given orally since low blood levels of the drug were observed in treated animals. But a study of the urinary excretion of the drug and its metabolites, following oral and intravenous administration, demonstrated the presence of large amounts of the metabolites of salicylamide, indicating that in fact the drug was well absorbed but rapidly metabolised.

The urinary excretion of many weakly acid and weakly basic drugs is dependent on the pH of the glomerular filtrate. Therefore any pharmacokinetic study based on urinary excretion data under conditions of uncontrolled urinary pH may be misleading particularly with respect to the biological half life. This is because tubular reabsorption of the weakly acid and basic drugs may be enhanced under acidic and alkaline pH respectively (Asatoor et al., 1965; Beckett et al., 1965; Orloff and Berliner, 1956; Nelson, 1961b; Portnoff et al., 1961). From what has been presented above, it may be apparent that a systematic and detailed knowledge of the pharmacokinetics of a drug is essential for its proper use, a view which has been supported by a number of authors (Kaplan, 1972; Nelson, 1961a; D.I.B., 1969; Wagner, 1961; Rossum, 1971; Wagner, 1971b).

Pharmacokinetic and biopharmaceutical approaches to the study of drug absorption, distribution and excretion are based on the concept of using mathematical models to account for the various events which occur. The various processes assumed to be taking place are defined by a number of differential equations. Because the solution of differential equations can be complex and tedious, it is clear that the process of determining pharmacokinetic parameters would be difficult without the help of computers. In fact, a number of pharmaceutical and pharmacological

researchers in the past avoided pharmacokinetic investigations because of the computational problems involved. Today the computations in pharmacokinetic investigations are straightforward because of the various computer programs which are readily available. Thus, even a researcher without much mathematical background can confidently handle pharmacokinetic investigations with the help of computers. Garrett et al. (1960) have presented the fundamentals and the philosophy of electronic analog computers. Analog computers have been used in the determination of various pharmacokinetic parameters and for testing the validity of proposed compartmental models (Beckett and Tucker, 1968a; Beckett et al., 1968b). Kirsten and Ross (1972) have published analog program circuits for simulation of multiple dosing kinetics, and variable dosing regimens were simulated using an analog computer by Kirschner et al. (1973). Although analog computers are excellent for the solution of differential equations, they are less accurate than digital computers for the determination of various pharmacokinetic parameters and rate constants. There are a number of digital computer programs available for iterative least squares fitting of plasma level data for the estimation of various pharmacokinetic constants and parameters. Berman (1965) used a program called SAAM to fit data to a desired physical or mathematical model by adjusting the parameter values of the model until the best fit was obtained. Other programs available are: BMD X85 (Series from Health Sciences Computer Facility, University of California at Los Angeles) and NONLIN (Metzler, 1968). An iterative least square method for finding the best fit to a multiexponential equation, written in ALGOL was reported by Koizumi et al. (1973). Niebergal et al. (1974) have recently published a computer program to calculate and predict plasma levels

during multiple dosing schemes. COMPT, a time-sharing program for nonlinear Regression Analysis of Compartment Models was recently reported by Pfeffer (1973).

It is clear from the preceding introduction that the kinetics of absorption, distribution and clearance of a drug in the body can be accounted for using mathematical models and that computer programs exist for fitting models to experimentally determined parameters. This in turn will generate a series of pharmacokinetic constants which can be used to account for, interpret and predict the behavior of a drug in the body.

CHAPTER II

LITERATURE REVIEW

Whilst reviewing the literature on the various aspects of pharmacokinetics, the author's attention was drawn to the absence of pharmacokinetic studies on the substituted succinimides, ethosuximide, methsuximide and phensuximide. These agents have been widely used as anti-convulsants in the treatment of petit mal epilepsy.

Miller and Long (1953a,b) of Park-Davis Laboratories had been attracted to the succinimide ring system in their search for new anti-convulsant drugs because of the structural similarities between the succinimide ring and the ring system of known anti-convulsant drugs (Figure 3). These workers synthesised a series of N-α,β-alkyl succinimides including ethosuximide, methsuximide and phensuximide. Chen et al. (1951, 1963) screened these substituted succinimides for anti-convulsant activity against leptazol-induced convulsions in rats. They found ethosuximide, methsuximide and phensuximide to be highly effective anti-convulsants in this test. Subsequent clinical studies demonstrated the clinical usefulness of these agents against petit mal epilepsy. A survey of the literature revealed that few pharmacokinetic or biopharmaceutical studies had been carried out on ethosuximide, methsuximide or phensuximide, in either animals or man. Most of the information which was available dealt with aspects of the physiological disposition of these agents rather than looking at their pharmacokinetics and biopharmaceutics. The literature available on these agents is reviewed below.

Ethosuximide.

Zimmerman and Burgemeister (1958) and Vossen (1958) reported the first clinical use of ethosuximide in petit mal epilepsy and ethosuximide

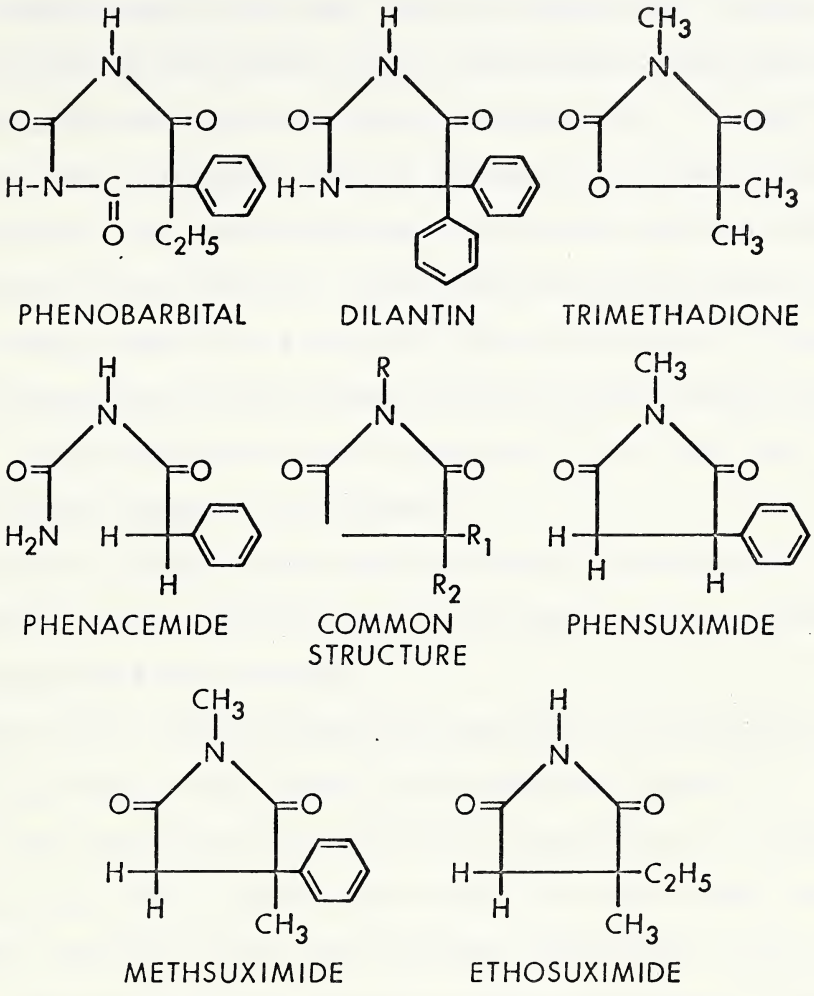

Figure 3. The chemical structures of substituted succinimides showing structural similarities with other anticonvulsants.

entered therapeutics as the drug of choice for petit mal epilepsy in 1961.

A limited study of the blood levels of ethosuximide in man was reported by Hansen and Feldberg (1964). These workers showed that the drug was rapidly absorbed following oral administration. A single dose of 750 mg. gave a peak plasma level of 15 mcg/ml. 2 - 4 hours following administration. The level of the drug in plasma then remained nearly the same for the next 24 hours. After a multiple dosing schedule of 250 mg. orally, three times a day for 12 days, they reported a plasma level of ethosuximide ranging between 35 and 55 mcg/ml. reached within 4 days. No determination of the biological half life or any other pharmacokinetic parameters was reported.

Dill et al. (1965) studied the physiological disposition of ethosuximide in man, monkey and rat, but the details of their findings were not published until recently.

Haerer et al. (1970) studied the blood levels of ethosuximide in epileptic patients. They reported levels averaging 40 mcg/ml. in children and young adults with petit mal, or mixed seizure, disorders. Buchanan et al. (1973) reported mean plasma levels of 33 mcg/ml. in five volunteers receiving a daily dose of 500 mg. ethosuximide, orally, for 9 days. The biological half life was reported to be 56 hours in this study.

The biological half life of ethosuximide has been shown to be species dependent. In man, the biological half life was reported to be 54 - 66 hours, in monkey around 22 hours and in rats about 12 hours (Chang et al., 1972). Also Sherwin and Robb (1972) have reported age related differences in the biological half life of ethosuximide. Buchanan et al. (1969) studied the blood levels of ethosuximide in

five children after a dose of Zarontin[R] syrup or Zarontin[R] capsules, orally. They reported that peak plasma levels occurred 3 - 4 hours following dosing. They reported the plasma half life of ethosuximide to be shorter in children than in adults (children, 30 hours; adults, 66 hours). These workers also found no differences between the total amount of ethosuximide absorbed from the syrup or capsule dosage forms.

Sherwin et al. (1973) monitored plasma levels of ethosuximide in 70 patients and showed that this resulted in improved control of the patient's epilepsy, when drug levels were maintained within specified limits.

Chang et al. (1972) summarized most of the information on the disposition of ethosuximide in a text on anti-epileptic drugs. The summary shows that ethosuximide is uniformly distributed throughout the body, following administration of the drug, with the exception of fat. This review also reports that the total urinary excretion of unchanged ethosuximide in man, monkey and rat was similar and ranged from 13 - 19 percent.

The metabolism of ethosuximide has been studied by several workers. Dill et al. (1965) took urine from rats and monkeys given ethosuximide, treated it with beta-glucuronidase and extracted the hydrolysate with chloroform. The chloroform extract was subjected to gas chromatography. Five peaks due to metabolites were found but they were not identified. Horning et al. (1973a) identified four monohydroxy metabolites of ethosuximide in the urine of rat and man. They also reported that in rat more unchanged drug than metabolites was present in plasma. In contrast, in man, in plasma a monohydroxy metabolite was present in larger amounts than the unchanged drug. In this study metabolites

were identified by gas chromatography-mass spectrometry.

Orton and Nicholls (1972a),using the reduction of hexobarbital sleeping time as a measure, claimed ethosuximide had the ability to induce hepatic microsomal enzymes. Synthesis of porphyrins was not affected in this study.

Nahorski (1972) reported that ethosuximide increased the brain/blood glucose ratio by stimulating the transport of glucose from blood to brain.

The survey shows that no detailed pharmacokinetic studies have been conducted on ethosuximide, most of the data is clinically orientated but not based on a rational pharmacokinetic approach to the use of the drug. It is clear that the drug has a long half life in some species and that all species studied metabolise the drug.

Methsuximide.

Methsuximide was introduced into clinical use by Zimmerman (1951). It differs from ethosuximide in structure (Figure 3). The presence of a phenyl substituent in the succinimide ring has been postulated to confer protection against electroshock convulsions. The α-methyl substituent is believed to confer protection against leptazol induced convulsions (Glazko and Dill, 1972). The drug has been reported to be effective in some patients with refractory petit mal epilepsy (Schmidt and Wilder, 1968) and it has been used occasionally in the treatment of psychomotor disorders (Livingston, 1966).

Glazko and Dill (1972)reviewed most of the available information on the physiological disposition of methsuximide. In rats, after oral administration the drug has been shown to be uniformly distributed

throughout the body tissues. In man, a single oral dose of 1200 mg of methsuximide was reported to produce a peak plasma level of 12.8 mcg/ml within one hour after administration of the drug. The reported plasma half life, in this study, was between 2 and 4 hours. It was noted that multiple dosing reduced the half life of the drug indicating that it may induce its own metabolism. Nicholls and Orton (1972), using ^{14}C-labelled methsuximide, showed that the drug was rapidly absorbed when given orally. These workers recovered 26 per cent of the administered radioactivity in the urine.

The metabolism of methsuximide has been studied by several workers. Glazko and Dill (1972), using gas chromatography, detected a number of metabolites of methsuximide in urine after derivatisation to form trimethylsilyl analogues, but none of the metabolites was identified. Muni et al. (1973) identified an N-demethylated metabolite of methsuximide using gas chromatography-mass spectrometry. Horning et al. (1973b) have identified three metabolites of methsuximide in the urine of rat, guinea pig and human following the administration of the drug. The metabolites were characterized as N,2-dimethyl-2(3,4-dihydroxy-1,5-cyclohexadien-1-yl)succinimide, and the two isomeric N,2-dimethyl-2(3-hydroxyphenyl)succinimide.

Orton and Nicholls (1972b) showed, in rats, that methsuximide could induce increased metabolism of hexobarbital and itself but did not affect liver synthesis of porphyrins. In contrast to this latter finding the N-demethylated metabolite had a well-defined porphyrinogenic effect on the liver of chick embryos.

Acute intoxication due to methsuximide is rare (Schulte and Good, 1966) but recently Karch (1973) reports an unusual case of an 18 year

old girl who had ingested an overdose of the drug. The girl was comatose 60 hours after ingestion of the methsuximide. During the comatosed condition, high levels of the N-demethylated metabolite of methsuximide were found in her plasma. The long lasting depression was attributed to the slowly excreted N-demethylated metabolite of methsuximide since the plasma half life of methsuximide was very short (3 hours).

It is clear from this literature survey that little systematic pharmacokinetic work has been performed on methsuximide. More attention appears to have been spent on the metabolism of the drug.

Phensuximide.

Zimmerman (1951) reported the first clinical use of phensuximide in the treatment of petit mal epilepsy. Most of the information on the physiological disposition of phensuximide has been summarized by Glazko et al. (1954) and Glazko and Dill(1972). Rats given 100 mg/kg of phensuximide, orally, attained a peak drug plasma level within 2 hours. The plasma half life was approximately 7 hours. In man, following a 1.8 g oral dose of phensuximide, the peak plasma levels recorded ranged between 10 - 19 mcg/ml, within four hours of dosing.

Administration of ^{14}C-labelled phensuximide to rats demonstrated that the drug was relatively uniformly distributed throughout the animals. Approximately 77 per cent of the administered radioactivity was recovered in the urine. The drug showed little, if any, protein binding and did not accelerate its own metabolism on multiple dosing.

Some work on the metabolism of the drug has been done. In rats, N-demethylation occurs to only a small extent. In man, the para-hydroxylated derivative of the drug has been reported as an important

metabolite in urine. Dudley et al. (1972) studied the metabolism of phensuximide in dog. They identified the N-demethylated metabolite of phensuximide, but it was present in only trace amounts, and a product of ring cleavage, levo-2-phenylsuccinic acid (14%) and N-methyl-α-(p-hydroxyphenyl) succinimide (5%) as the third metabolite.

The review of the literature revealed that negligible pharmacokinetic information was available on ethosuximide, methsuximide and phensuximide. Hence, it was felt that a pharmacokinetic and a biopharmaceutical investigation of the three succinimides would help in attaining a better understanding of the absorption, distribution and metabolism of these drugs. It would also help to justify the dosage forms and dose regimens usually employed, empirically, in clinical practice. Therefore a systematic pharmacokinetic investigation of the succinimides was undertaken.

Assay Methods for the Determination of Succinimides in Biological Fluids and Tissue Homogenates

A sensitive, selective, reproducible, rapid method of estimation of the drug is essential for any pharmacokinetic or biopharmaceutical study. Although methods involving measurements of the radioactivity of labelled compounds are highly sensitive and can be used when no other methods are available for the quantitative analysis of a drug, they are not generally suitable for pharmacokinetic or biopharmaceutical studies. This is because one only measures the 'label' and cumbersome seperation techniques may be necessary to differentiate the labelled drug from its labelled metabolites. Unless this is done, inaccurate estimates of the pharmacokinetic parameters will be obtained because each metabolite has its own volume of distribution and other pharmacokinetic parameters. Thus, Chang et al. (1972) reported that the half-life of labelled ethosuximide, determined by measuring total radioactivity in plasma, was three times longer than the half-life when gas chromatography was used to estimate the drug.

There is not a great deal of information in the literature on the estimation of these anticonvulsant drugs. Phenyl substituted anticonvulsant drugs have been determined colorimetrically by nitration of the phenyl ring, followed by reduction and diazotization of the primary amino groups (Huisman, 1966), but this method is non-specific and time consuming for large numbers of analyses.

Phensuximide has been determined in blood and urine by fluorimetry, a method based on the fact that the drug forms a fluorescent product with iodine (Glazko et al., 1954). But again this method was time consuming and, likely, non-specific.

A semi-quantitative thin layer chromatographic method was reported for ethosuximide, methsuximide and phensuximide in blood (Gardner-Thorpe et al., 1971).

The dried thin layer chromatography silica gel-coated plates were examined under U.V. light to detect methsuximide and phensuximide. Ethosuximide was detected by spraying the plates with mercurous nitrate solution. Hansen and Feldberg (1964) used a U.V. method for the estimation of ethosuximide in blood. The method depended on measuring the absorbance of a chloroform extract of the blood sample at 218 nm. Blanks often gave high readings and barbiturates interfered with the analysis. None of the methods discussed so far met the requirements of a pharmacokinetic study.

Attention was then turned to the use of chromatography. Gas-liquid chromatography (GLC) has been widely used for the analysis and detection of drugs in body fluids and tissues (Jain and Kirk, 1967). A number of authors have reported the use of gas chromatography in the simultaneous determination of a number of anticonvulsant drugs (Solow and Green, 1972; Roger et al., 1973; Pippenger and Gillen, 1969). Dill et al. (1965) reported a method in which a chloroform extract of acidified blood and urine was chromatographed using α,α-dimethyl-β-methyl succinimide as an internal standard, on a 6-foot column packed with 5% XE-60 on Gas Chrom Z at 155°C. 5% OV-17 on Gas Chrom Q, (100/200 mesh) has been used (Solow and Green, 1971; Solow and Green, 1972) to determine ethosuximide in blood following derivatization of ethosuximide with tetramethylammonium hydroxide at 100°C. Phensuximide and methsuximide have also been determined, on the same column, by temperature programming between 210°C and 230°C. Ethosuximide and phensuximide (Kleijn et al., 1973) have been assayed in plasma and urine by GLC on 3% OV-17 at 125°C on Gas-Chrom Q 80-100 mesh. Naphthalene was used as the internal standard for ethosuximide and di-n-butyl phthalate as the

internal standard for phensuximide.

GLC appeared to offer the best chance of finding a satisfactory method of analysis, for ethosuximide, methsuximide and phensuximide, for a pharmacokinetic study. Accordingly this was chosen as the method to be used in the studies which follow.

CHAPTER III

AIMS AND OBJECTIVES OF THE INVESTIGATION

The objectives of the studies conducted in the present investigation were set as follows:

1. To examine existing GLC methods for estimating ethosuximide, methsuximide and phensuximide and to find or develop a method which would estimate these drugs, in small quantities of blood, plasma, urine or tissue homogenates, rapidly and reproducibly.

2. To investigate and compare the pharmacokinetic properties of ethosuximide, methsuximide and phensuximide following intravenous administration to rats.

3. To look for evidence of dose dependency in the pharmacokinetics of ethosuximide following intravenous administration of the drug to rats.

4. To study the multiple dose kinetics of ethosuximide following oral administration of the drug to rats.

5. To apply the pharmacokinetic findings in a study of the systematic availability of ethosuximide in rats, following oral administration of the drug.

6. To examine the effect of various pharmaceutical adjuvants on the systematic availability of ethosuximide in rats, following oral administration of the drug.

7. To investigate the urinary excretion of ethosuximide in rats under conditions of controlled urinary pH or in the presence of probenecid.

CHAPTER IV

EXPERIMENTAL METHODS AND RESULTS

1. ANALYTICAL METHODOLOGY

a. Gas-Liquid Chromatographic Procedure.

A Perkin-Elmer[a] 990, or a Hewlett-Packard[b] 5700 A, gas chromatograph equipped with a dual flame ionization detector, a strip chart recorder and an electronic integrator[c] was used for the analysis of the succinimides in this study. Seven spiral glass columns (4 ft. x 6 mm. O.D.), each with a different liquid stationary phase, were prepared using chromosorb W(AW-DMCS), 80-100 mesh, as the solid support. The liquid stationary phases used were: XE-60 (Nitrile Silicone Gum) 2.5%, XE-60 5%, OV-1 (High Purity Nonpolar Dimethyl Silicone Gum) 3%, OV-17 (High Purity Polar Phenyl Methyl Silicone) 3%, OV-25 (High Purity Polar Phenyl Methyl Silicone) 3%, OV-210 (High Purity Polar Trifluoropropyl Silicone) and 3% OV-225 (High Purity Highly Polar, Cyanopropyl Phenyl Silicone). The columns were conditioned at 250°C for 24 hours prior to use in the analysis. Helium was used as carrier gas in all cases. One microliter (mcl) of a solution (1 mg/ml) of a particular drug, dissolved in chloroform, containing a suitable internal standard was injected into the gas chromatograph using a 10 mcl syringe. The settings for column temperature, detector temperature, injection port temperature, hydrogen and air pressure, and helium flow rate were varied for each injection. The resulting peaks, due to drug and internal standard, were examined with respect to retention time, peak width, peak height, symmetry of the peak, degree of tailing, and the

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[a] Perkin-Elmer, Norwalk, Connecticut.
[b] Hewlett-Packard, San Diego, California.
[c] Dinfortronics CSR-208, Columbia Scientific Industries, Texas.

position of the peaks relative to the solvent peak. (Two consecutive peaks were considered resolved when the ratio $(T_2 - T_1)/(W_2 - W_1)$ was greater than 1; where T_2 and T_1 represent the retention times of the drug, and the internal standard, and W_2 and W_1 are the respective peak widths at the base.) The column and the conditions which resulted in the best peak resolution giving symmetrical narrow peaks with maximal peak height were selected for the routine analysis of the succinimides in the blood, urine, and tissue homogenates.

It was soon apparent that XE-60 was not a suitable liquid phase. Peak resolution was poor, considerable tailing occurred and the peaks for the drugs emerged on the solvent front. Essentially similar results were obtained with OV-1, OV-17 and OV-25. OV-210 gave better results but the best column was 3% OV-225. This gave good resolution of ethosuximide, methsuximide and phensuximide. It was found empirically that biphenyl was a good internal standard for ethosuximide and that methsuximide and phensuximide were excellent internal standards for each other. The conditions for analysis were optimised for each drug. These are summarized in Table I.

b. Ethosuximide.

The efficiency of the column was found to be best at a linear carrier gas velocity of 40 ml/min. The maximal number of theoretical plates, found at this gas velocity, was 1555 with a corresponding height equivalent to one theoretical plate of 0.1364 (HETP). N, the number of theoretical plates and HETP were calculated from equations

TABLE I

The optimal conditions for the gas chromatographic analysis of succinimides in chloroform extracts of blood, urine and tissue homogenates.

Conditions	Perkin-Elmer 990	Hewlett-Packard 5700 A
Column	4 ft x 6 mm O.D. glass	4 ft x 6 mm O.D. glass
Solid Support	Chromosorb W (AW-DMCS) 80-100 mesh	Chromosorb W (AW-DMCS) 80-100 mesh
Staionary phase	3% OV-225	3% OV-225
Helium flow rate	37 ml/min	40 ml/min
Air inlet pressure	40 psig	60-13 psig
Hydrogen inlet pressure	24 psig	17.5 psig
Oven Temperature[a]	150°C	150°C
Injection port[a] temperature	200°C	200°C
Detector temperature[a]	200°C	200°C
Chart Speed	0.5 in/min	0.25 in/min
Retention time Ethosuximide	5.6 min	5.6 min
Retention time Biphenyl	3.8 min	3.8 min
Retention time Methsuximide	3.7 min	3.7 min
Retention time Phensuximide	5.5 min	5.5 min

[a]For the analysis of methsuximide and phensuximide all the conditions were similar except the oven temperature detector temperature and injection port temperature were raised by 50°C.

$$N = 16\ (x/y)^2 \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (32)$$

$$HETP = L/N \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (33)$$

where N was number of theoretical plates, x was the distance from the point of injection to the center of the peak on the trace, y was the peak width at the base, and L was the length of the column. A typical chromatogram is shown in Figure 4.

(i) Calibration Curve for Ethosuximide in Chloroform.

Standard solutions of ethosuximide[a] in chloroform in the range 5-100 mcg/ml were prepared. One ml of each respective standard solution was pipetted into a 15 ml conical glass centrifuge tube containing 20 mcg of biphenyl in 50 mcl of chloroform as internal standard. The sides of the tube were washed down carefully with about 5 ml of fresh chloroform and the contents well mixed. The chloroform was then evaporated on a water bath at 65°C to about 100 mcl volume. One to 5 mcl samples of the concentrate were injected into the column of the gas chromatograph maintained at the specified conditions in Figure 4. The peak areas for biphenyl and ethosuximide were determined by the electronic integrator. The attenuator setting of the gas chromatograph was fixed at 10 x 1 and the integrator attenuator setting was varied to keep the detector response within the recorder paper. Each determination was made in triplicate. A calibration curve was then constructed by plotting the ratio of peak area of the ethosuximide to the peak area of biphenyl against the known amounts of ethosuximide in the chloroform solution. The data obtained for the calibration curve of

[a] Zarontin®, Lot C427606, Park, Davis and Company, Ltd., Brockville, Ontario.

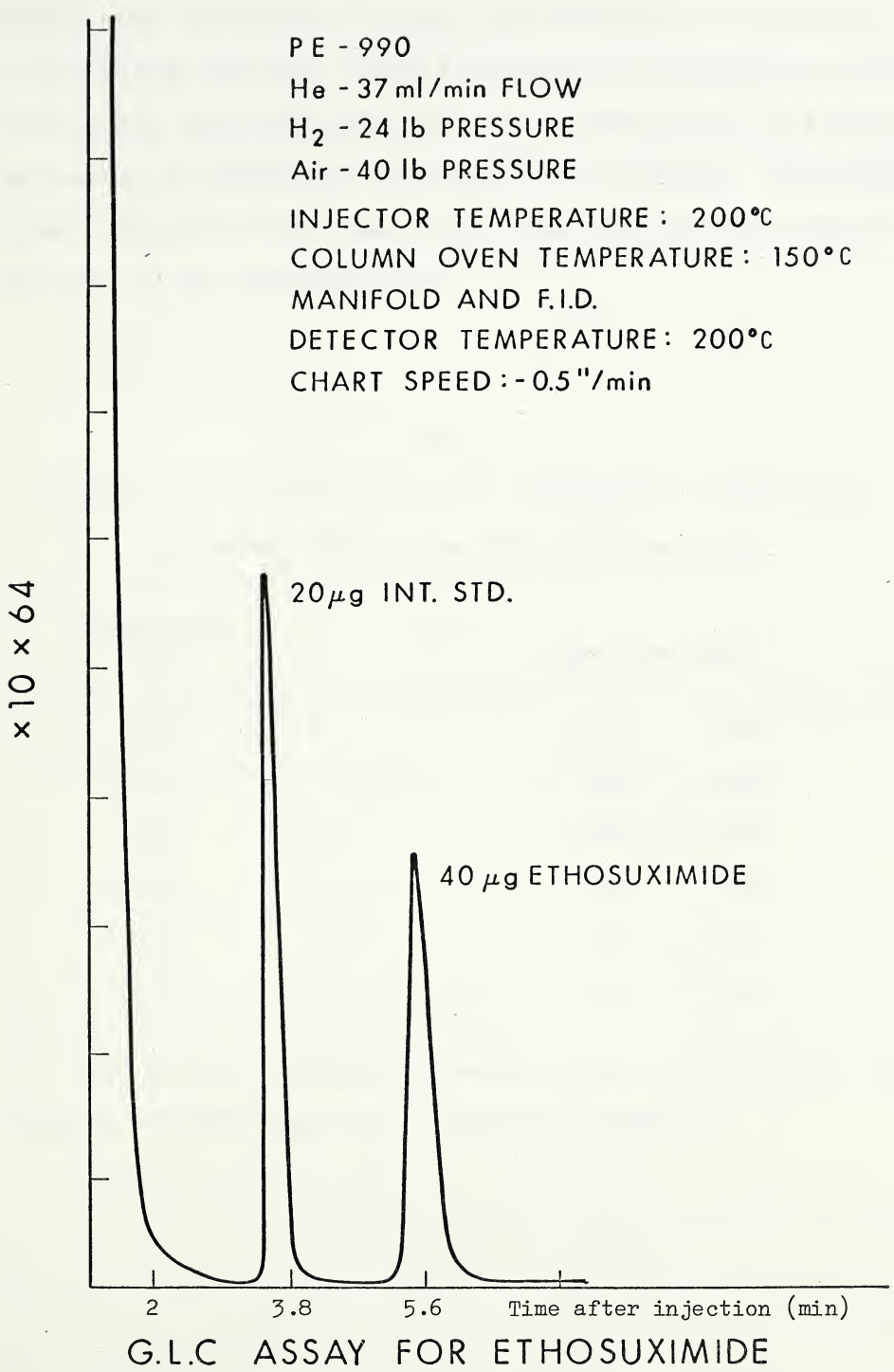

Figure 4. Typical chromatogram of a plasma extract containing ethosuximide with biphenyl as internal standard. Instrument: Perkin-Elmer 990 Gas Chromatograph.

ethosuximide in chloroform for each gas chromatograph are presented in Table II and Table III. Figure 5 and Figure 6 illustrate the linear relationship between the peak area ratio of ethosuximide and biphenyl and amounts of ethosuximide obtained on each instrument. Calibration curves prepared at later times did not show any significant change in the slope of the calibration line.

TABLE II

Data for the calibration curve for ethosuximide in chloroform

Instrument: Perkin-Elmer 990 Gas Chromatograph

Ethosuximide (mcg)	Peak Area Ratio[a]
5.0	0.119 ± 0.002
10.0	0.242 ± 0.022
20.0	0.481 ± 0.034
50.0	1.192 ± 0.169
100.0	2.749 ± 0.243
150.0	4.369 ± 0.169

[a]Mean of three determinations with standard deviation Slope = 0.0292, Intercept = -0.0689, Correlation Coefficient = 0.995.

TABLE III

Data for the calibration curve for ethosuximide in chloroform

Instrument: Hewlett-Packard 5700 A Gas Chromatograph

Ethosuximide (mcg)	Peak Area Ratio[a]
0.1	0.002 ± 0.003
0.5	0.011 ± 0.001
1.0	0.022 ± 0.003
10.0	0.294 ± 0.029
30.0	0.897 ± 0.144
60.0	1.834 ± 0.056
100.0	3.313 ± 0.234

[a]Mean of three determinations with standard deviation Slope = 0.0327, Intercept = -0.0316, Correlation Coefficient = 0.9962.

Having shown that it was possible to estimate the drug in chloroform, the next step was to attempt to estimate the drug in blood, plasma, urine or tissue homogenates. Solvent extraction was used successfully in this work.

(ii) General Extraction Procedure.

Extraction of ethosuximide from blood, urine, or tissue homogenates: One ml of urine, or 50 mcl of blood, or 50 mcl of tissue homogenate in saline, was pipetted[a] into a 15 ml conical glass stoppered centrifuge tube

[a] Oxford Laboratories Micro Pipett, Fostercity, California.

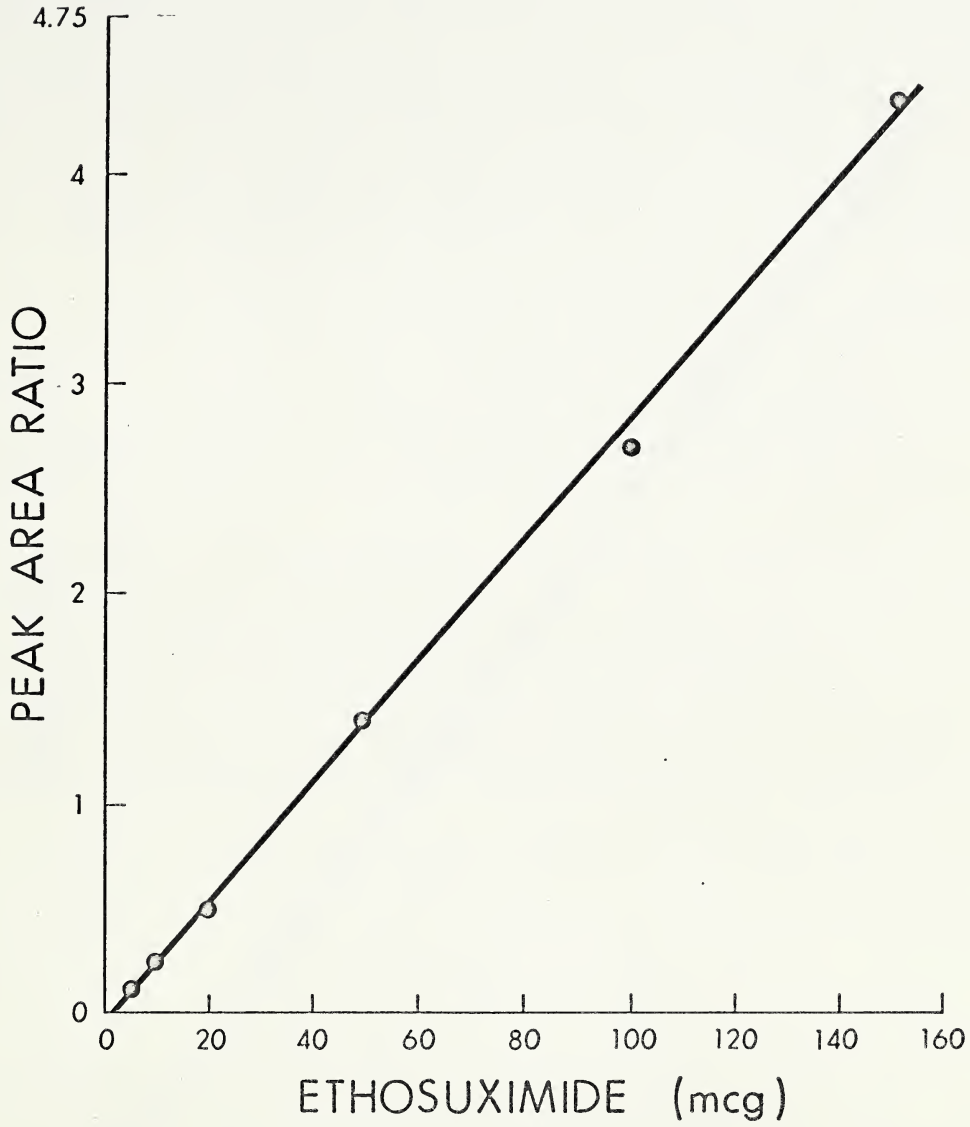

Figure 5. GLC calibration curve for ethosuximide in chloroform. Instrument: Perkin-Elmer 990 Gas Chromatograph.

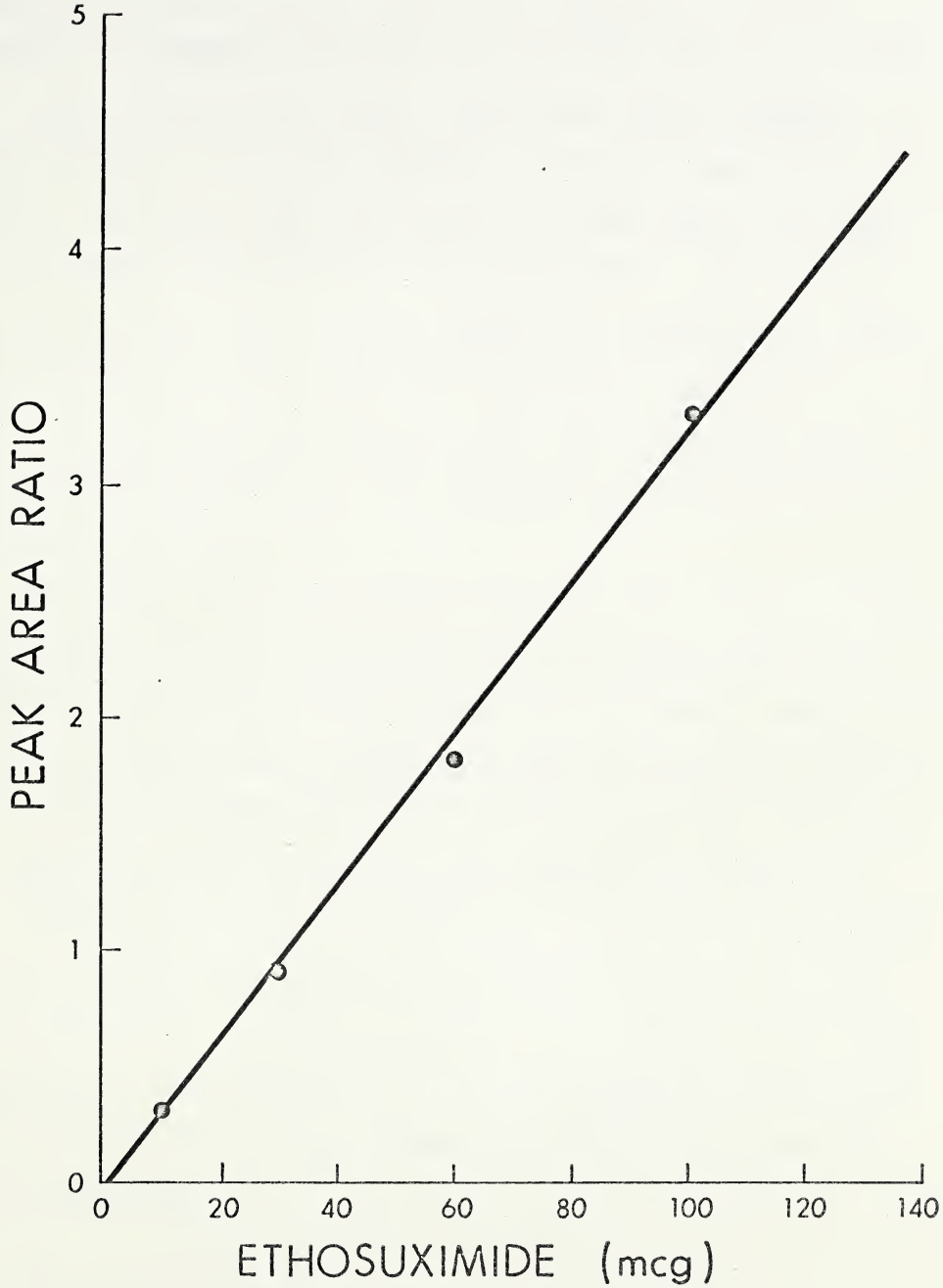

Figure 6. GLC calibration curve for ethosuximide in chloroform. Instrument: Hewlett-Packard 5700 A Gas Chromatograph.

containing 20 mcg of biphenyl in 50 mcl of chloroform as internal standard. The contents of the tube were acidified with 1 ml of 1 N HCl and 10 ml of chloroform was added. The tube was tightly stoppered, clamped in place in a box, and shaken on a mechanical shaker for 20 minutes. The tube was then centrifuged in an Adams Dynack centrifuge model CT-1300[a] at speed setting of 90 for 20 minutes. The chloroform layer was withdrawn using a pasteur pipette and transferred to a clean conical centrifuge tube and evaporated to a volume of approximately 100 mcl on a water bath maintained at 65°C. One to 5 mcl of concentrated chloroform extract was then chromatographed under the specified conditions in (i) above.

The absolute recovery of ethosuximide from blood, urine or tissue homogenates was determined by extracting in triplicate a known amount of each drug from 'blank' blood, urine or tissue homogenate samples using the procedure described above. The amount recovered was compared with the amount found in a chloroform solution of each drug containing the same amount of drug, using the same procedure, at the same time.

The extraction procedure employed was very efficient. The absolute recovery of ethosuximide from blood was found to be 98-100%. The recovery from 'blank' rat urine was 96-103%. The recovery from the tissue homogenates or feces was 95-101%. The percent recovery of ethosuximide from rat blood, urine or tissue homogenates is presented in Tables IV, V, VI respectively.

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[a]Clay Adams, Becton, Dickerson and Company, Parsippany, New Jersey.

TABLE IV

Recovery of ethosuximide from blank blood of rat

Ethosuximide (mcg)	Recovered (mcg)	Recovery[a] (%)
5.0	4.91	98.25 ± 3.77
20.0	19.93	99.65 ± 3.50
40.0	39.85	99.63 ± 3.70
80.0	80.52	100.65 ± 3.95
100.0	99.95	99.95 ± 3.25

[a]Mean of three determinations with standard deviation.

TABLE V

Recovery of ethosuximide from blank urine

Ethosuximide (mcg)	Recovered (mcg)	Recovery[a]
5.0	4.789	95.78 ± 2.8
20.0	20.52	102.6 ± 3.5
40.0	39.756	99.39 ± 3.3
80.0	79.99	99.98 ± 3.1
100.0	101.25	101.25 ± 3.8

[a]Mean of three determinations with standard deviation.

TABLE VI

Recovery of 10 mcg ethosuximide added to 1 g/ml of various tissue homogenates or feces or 1 ml of water

Tissue samples	Ethosuximide per ml of homogenate (mcg)	Recovered (mcg)	Recovery[a] (%)
Liver	10.0	9.74	97.40 ± 3.1
Lung	10.0	9.56	95.60 ± 3.8
Kidney	10.0	9.88	98.82 ± 3.5
Heart	10.0	10.09	100.90 ± 3.7
Muscle	10.0	9.85	98.52 ± 3.7
Fat	10.0	9.64	96.41 ± 3.2
Brain	10.0	9.96	99.55 ± 3.2
Feces	10.0	10.08	100.80 ± 3.7
Water	10.0	10.10	101.00 ± 3.5

[a]Mean of five replicates with standard deviation.

(iii) Calibration Curves for Ethosuximide in Urine and Blood.

Normal urine and blood was collected from rats which had never received any drug. Standard solutions of ethosuximide were prepared to contain 5-100 mcg of drug in 1 ml of urine, or 50 mcl of blood plasma. One ml of urine standard solution, or 50 mcl of blood standard solution, was extracted according to the extraction procedure for each drug and 1 to 5 mcl of the concentrated chloroform extract was chromatographed in the manner described earlier. The peak area ratio of respective drug and internal standard was plotted against the known amounts of

respective drug in blood or urine sample. Each determination was made in triplicate. A slight difference between the slopes of the calibration lines of ethosuximide in urine and blood was noticed as illustrated in Figure 7.

These calibration curves were repeated every 3 months but they remained stable during the period of this study.

(iv) Reproducibility of the Extraction Procedure.

The reproducibility of the extraction procedure from blood, urine, or tissue homogenates was confirmed by extracting 10 urine, blood or tissue homogenate samples each containing 10 mcg/ml of ethosuximide along with the internal standard and analyzing for the drug.

The measure of the reproducibility of the extraction procedure in terms of the standard deviation of ten analyses of blood, urine or tissue samples each containing 10 mcg per ml or per gram of tissue was ± 2.0%.

(v) Precision of the Assay.

The precision of the estimation procedure was determined by giving 10 successive injections of drug solution in chloroform together with the internal standard from the same solution during a 2 hour period. The amounts of drug calculated from each injection were subjected to statistical analysis and the precision was expressed as the coefficient of variation of the mean. The precision of the method,expressed as the coefficient of variation of the mean of 10 consecutive determinations, was found to be 2.02%.

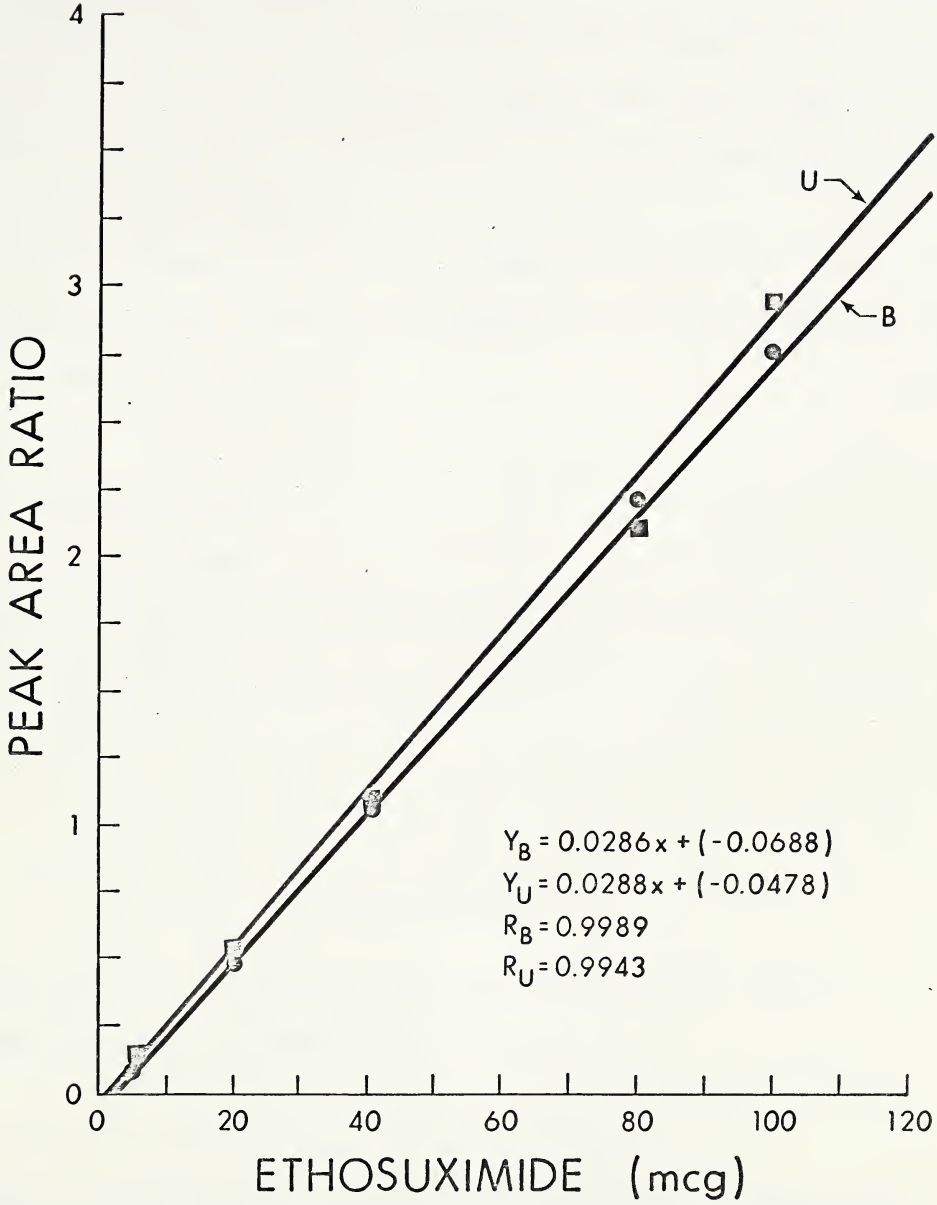

Figure 7. GLC calibration curve for ethosuximide in urine (U) and blood (B). Y_B = observed peak area ratio ethosuximide: biphenyl in blank blood, Y_U = observed peak area ratio ethosuximide: biphenyl in blank urine, R_B = correlation coefficient for ethosuximide assay in blood extract, R_U = correlation coefficient for ethosuximide in urine extract.

(vi) Deterioration of Samples.

In order to determine any deterioration of the drug prior to extraction after collection of samples, samples were kept at room temperature for 24 hours or refrigerated at 4^0C for two weeks and analyzed at suitable intervals.

No deterioration of ethosuximide in blood, urine or tissue homogenates occurred when stored in a refrigerator at 4^0C for two weeks or at room temperature for 24 hours.

(vii) Interfering Peaks.

No interfering peaks were found in the blank extracts of blood or urine. Figure 8 shows the good peak resolution of ethosuximide and biphenyl from blood and urine extracts.

c. Methsuximide.

The procedures and apparatus used have been listed in Table I and described under b. Ethosuximide.

(i) Calibration Curve for Methsuximide in Chloroform.

The procedure was the same as described above except methsuximide[a] was substituted for ethosuximide. The internal standard used was 20 mcg of phensuximide in 50 mcl of chloroform and the peak area ratio plotted

[a] Celontin^R, Lot C427835, Park, Davis and Company, Ltd., Brockville, Ontario.

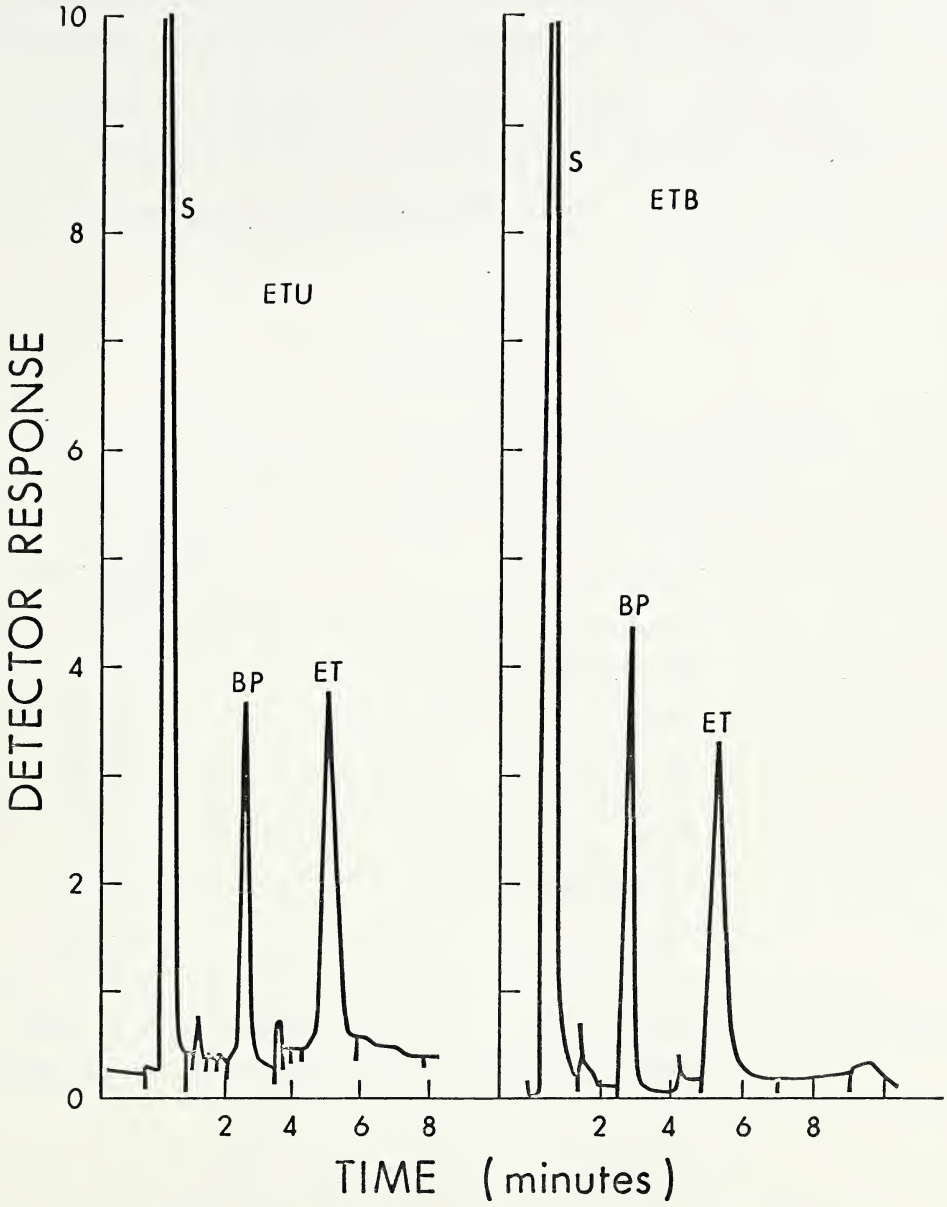

Figure 8. Typical chromatogram of urine extract (ETU) and blood extract (ETB) for ethosuximide. S = Solvent (Chloroform) peak, BP = Biphenyl. the internal standard, and ET = Ethosuximide. Instrument: Hewlett-Packard 5700 A Gas Chromatograph. Markings at the base of peak shows start or an end of the integration.

was that due to methsuximide/phensuximide against known amounts of methsuximide. The data for the calibration curve of methsuximide in chloroform are presented in Table VII. Figure 9 depicts the linear relationship between the peak area ratio of methsuximide to phensuximide and amounts of methsuximide.

TABLE VII

Data for the calibration curve of methsuximide in chloroform

Instrument: Hewlett-Packard 5700 A Gas Chromatograph

Methsuximide (mcg)	Peak Area Ratio[a]
5.0	0.265 ± 0.004
10.0	0.528 ± 0.016
30.0	1.614 ± 0.122
60.0	3.345 ± 0.027
100.0	5.405 ± 0.325
200.0	10.410 ± 0.536

[a]Mean of three determinations with standard deviation Slope = 0.0523, Intercept = 0.0866, Correlation Coefficient = 0.9978.

(ii) Extraction Procedure.

This has been described under b. Ethosuximide above. The procedure used for blood or urine was similar except that 20 mcg of phensuximide in 50 mcl of chloroform was used as internal standard. The absolute recovery of methsuximide from blank urine samples of rat was 101-106% as

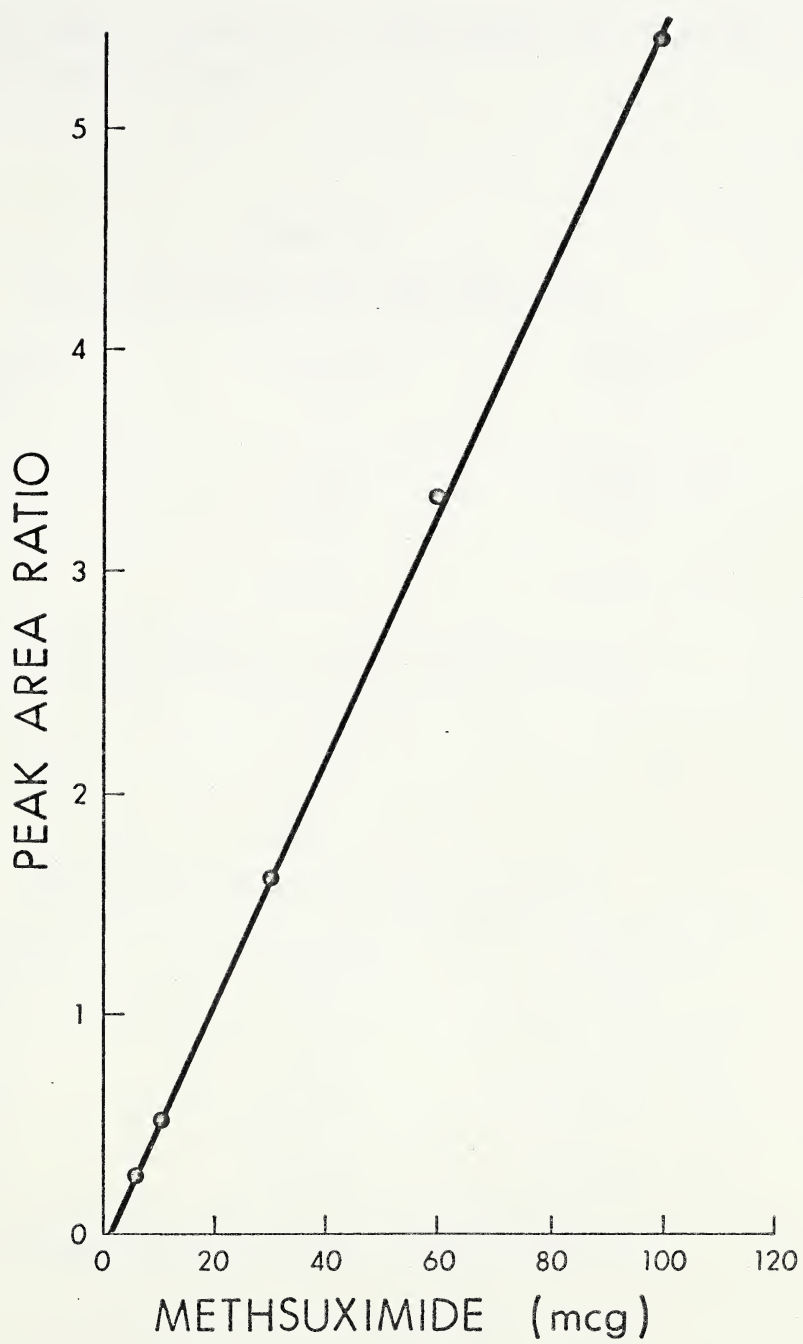

Figure 9. GLC calibration curve for methsuximide in chloroform. Instrument: Hewlett-Packard 5700 A Gas Chromatograph.

shown in Table VIII. The recovery of methsuximide from rat blood was 93-107% as shown in Table IX.

TABLE VIII

Recovery of methsuximide from blank urine.

Methsuximide (mcg)	Recovered (mcg)	Recovery[a] (%)
1.0	1.068	106.820 ± 6.5
10.0	10.656	106.560 ± 6.5
100.0	101.158	101.158 ± 4.5

[a]Mean of three determinations with standard deviation.

TABLE IX

Recovery of methsuximide from blank blood.

Methsuximide (mcg)	Recovered (mcg)	Recovery[a] (%)
1.0	0.929	92.98 ± 10.2
10.0	10.708	107.08 ± 5.2
100.0	102.268	102.26 ± 3.5

[a]Mean of three determinations with standard deviation.

(iii) Calibration Curve for Methsuximide in Blood.

The method described under b. Ethosuximide (iii) was used. Phensuximide was used as internal standard.

(iv) Reproducibility of the Extraction Procedure.

This was found to be similar to ethosuximide.

(v) Precision of the Assay.

This was determined as described previously under ethosuximide. The precision of the assay was similar to the ethosuximide assay.

(vi) Deterioration of Samples.

This was not studied. Samples were stored at $4^{0}C$ until assayed. The storage interval was kept to a minimum.

(vii) Interfering Peaks.

No interfering peaks were found in blank extracts of blood (Figure 10).

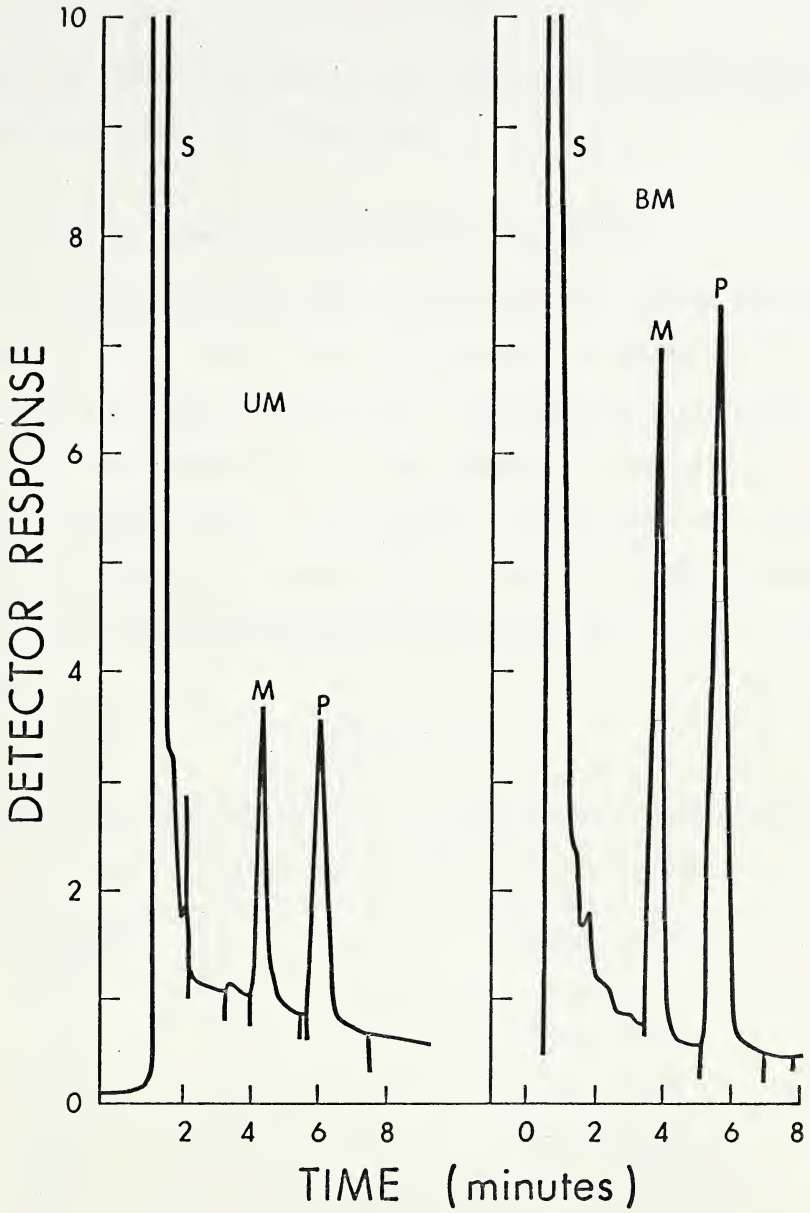

Figure 10. Typical chromatogram of urine extract (UM) and blood extract (BM) for methsuximide and phensuximide. S = Solvent (Chloroform) peak, M = Methsuximide, P = Phensuximide.
Instrument: Hewlett-Packard 5700 A Gas Chromatograph.

d. Phensuximide

The procedures and apparatus used have been listed in Table I and described under b. Ethosuximide.

(i) Calibration Curve for Phensuximide in Chloroform.

The procedure was the same as described above, except phensuximide[a] was substituted for ethosuximide. The internal standard was 20 mcg of methsuximide in 50 mcl of chloroform. The peak area ratio phensuximide/methsuximide was plotted against known amounts of phensuximide. The data for the calibration curve of phensuximide in chloroform are presented in Table X. Figure 11 illustrates the linear relationship between the peak area ratio of phensuximide to methsuximide and amounts of phensuximide.

TABLE X

Data for the calibaration curve of phensuximide in chloroform

Instrument: Hewlett-Packard 5700 A Gas Chromatograph

Phensuximide (mcg)	Peak Area Ratio[a]
5.0	0.226 ± 0.003
10.0	0.452 ± 0.012
30.0	1.367 ± 0.019
60.0	2.767 ± 0.040
100.0	4.532 ± 0.032
200.0	9.103 ± 0.103

[a]Mean of three determinations with standard deviation Slope = 0.0455, Intercept = 0.0042, Correlation Coefficient = 0.9999.

[a]Milontin[R], Lot C42764, Park, Davis and Company, Ltd., Brockville, Ontario.

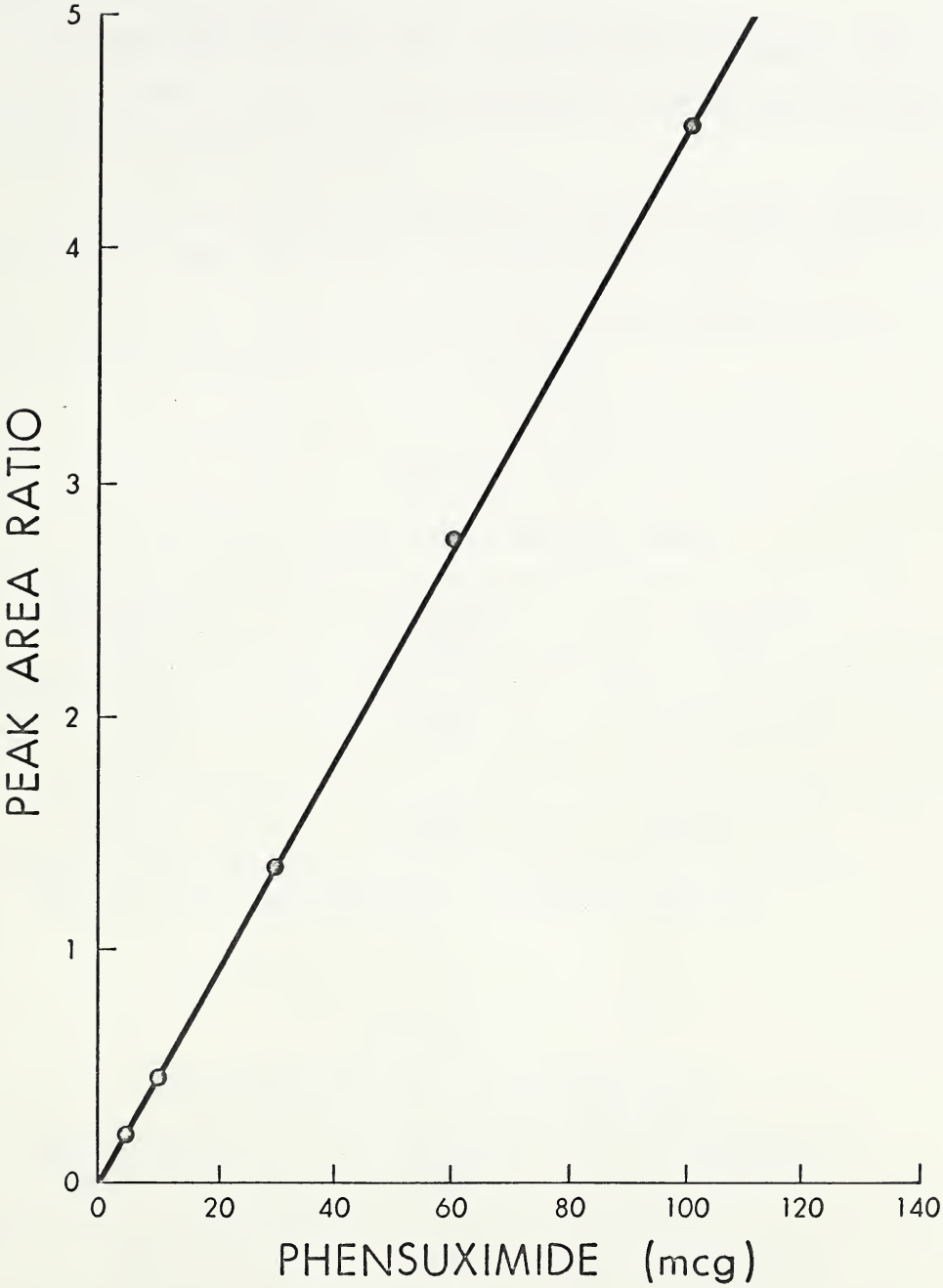

Figure 11. GLC calibration curve for phensuximide in chloroform. Instrument: Hewlett-Packard 5700 A Gas Chromatograph.

(ii) Extraction Procedure.

This has been described under b. Ethosuximide (ii) above. The procedure differed only in the use of 20 mcg of methsuximide in 50 mcl chloroform as internal standard.

The absolute recovery of phensuximide from rat blood was found to be between 91-100%. The recovery of phensuximide from rat 'blank' urine was 85-100%. Tables XI and XII list the percentage recovery of phensuximide from blood and urine respectively.

TABLE XI

Recovery of phensuximide from blank blood.

Phensuximide (mcg)	Recovered (mcg)	Recovery[a] (%)
1.0	0.911	91.07 ± 7.8
10.0	9.261	92.60 ± 6.8
100.0	100.899	100.89 ± 4.1

[a]Mean of three determinations with standard deviation.

TABLE XII

Recovery of phensuximide from blank urine.

Phensuximide (mcg)	Recovered (mcg)	Recovery[a] (%)
1.0	0.846	84.26 ± 15.4
10.0	9.330	93.30 ± 6.7
100.0	100.283	100.28 ± 4.5

[a]Mean of three determinations with standard deviation.

(iii) Calibration Curve for Phensuximide in Blood.

The method described under b. Ethosuximide (iii) was used. Methsuximide was used as internal standard.

(iv) Reproducibility of the Extraction Procedure.

This was found to be similar to ethosuximide.

(v) Precision of the Assay.

Determined as described previously under ethosuximide. The precision of the assay was similar to the ethosuximide assay.

(vi) Deterioration of Samples.

This was not studied. Samples were stored at 4°C until assayed. The storage interval was kept to a minimum.

(vii) Interfering Peaks.

No interfering peaks were found in blank extracts of blood (Figure 10).

e. Confirmation of the Identity of the Peaks

The GLC peaks obtained from the injection of chloroform extracts of blood, urine or tissue homogenates at the retention times of 5.5 min (150°C) and 3.7 min and 5.5 min at 200°C, were identified by GLC/mass spectrometry as unchanged, ethosuximide, methsuximide and phensuximide respectively. Figure 12 shows authentic mass spectra of the three drugs, which were identical to literature spectra (Locock and Coutts, 1970).

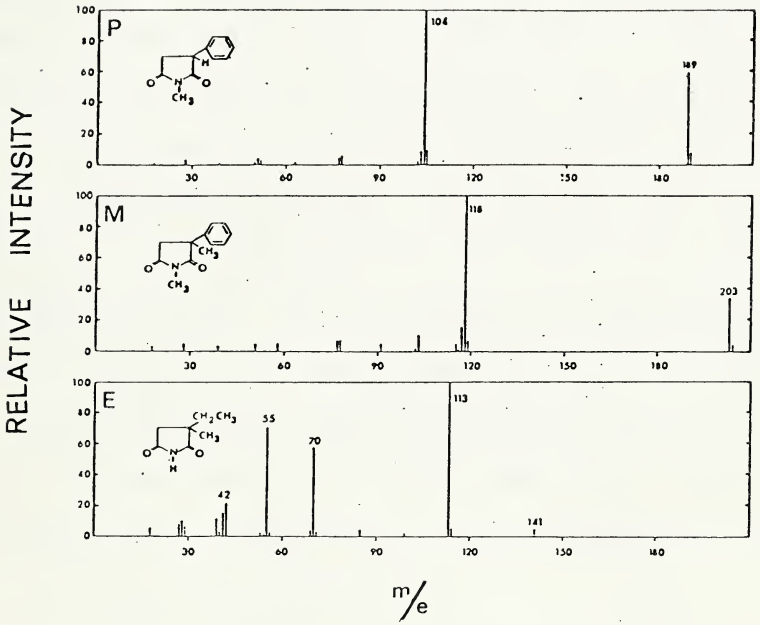

Figure 12. Mass spectrometric identification of GLC peak due to parent drug. E. = ethosuximide, M = methsuximide, P = phensuximide.

2. PROTEIN BINDING STUDY

The equilibrium dialysis apparatus for study of protein binding was set up in the usual manner (Patel and Foss, 1965). The two chambers of the plexiglass dialysis cells were separated by a washed seamless cellulose[a] membrane. 10 ml of 4% bovine serum albumin[b]-fraction V (BSA) in Krebs solution, adjusted to pH 7.4 was added to one chamber. 10 ml of Krebs solution (pH 7.4) containing 20.0, 200.0 or 1000.0 mcg of ethosuximide was added to the other chamber. Three dialysis cells were used for each concentration of ethosuximide studied, and three dialysis cells with BSA served as controls. The cells were then agitated on a shaker bath at 37°C until equilibrium was established; this took approximately 20 hours. The concentration of ethosuximide in the two chambers of the test cells was determined at the end of the equilibration time using 100 mcl samples assayed as described previously. The protein binding of ethosuximide was found to be negligible. Table XIII shows the concentration of ethosuximide in the two chambers of dialysis cell after 20 hours equilibration at 37°C.

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[a] Fisher Scientific Co. Ltd., Pittsburgh, Pa.
[b] Armour Pharmaceutical Co., Chicago, Ill.

TABLE XIII

Ethosuximide concentration in chamber A and B of dialysis cell after 24 hours equilibrium time at 37°C .

Initial Concentration of Ethosuximide in Chamber A (mcg/ml)	Ethosuximide Concentration in Chamber A[b] at Equilibrium (mcg/ml)	Ethosuximide Concentration in Chamber B[c] at Equilibrium (mcg/ml)
20.0	8.99 ± 0.35[a]	9.85 ± 0.45
200.0	101.25 ± 3.50	99.95 ± 2.9
1000.0	498.55 ± 10.50	501.15 ± 10.2

[a] Mean of three replicates.

[b] 1×10^{-4}M (4%) Protein solution (10 ml) BSA.

[c] 10 ml of pH 7.4 Buffer.

3. DRUG DISTRIBUTION BETWEEN PLASMA AND RED BLOOD CELLS

This study was undertaken to find out if it would be satisfactory to use whole blood samples instead of plasma samples to measure plasma levels of ethosuximide.

Four healthy Wistar rats were anesthetized with urethane (1.25 g/kg, intraperitoneally). The femoral vein, femoral artery and the trachea were cannulated. This procedure is described in more detail later. An intravenous injection of ethosuximide solution containing 40 mg of drug in dextrose/saline injection USP was administered through the femoral vein. 50 mcl whole blood samples and 50 mcl plasma samples for analysis for ethosuximide and approximately 100 mcl blood samples for hematocrit determination were collected prior to, and at various time intervals after, administration of the drug. Samples of blood for hematocrit determination were collected in standard hematocrit tubes, centrifuged, the heights of the separated layers measured and the hematocrit determined.

No change in the hematocrit was found following the intravenous injection of ethosuximide to rats during the study period. The ratio of the concentration of ethosuximide in plasma and whole blood was 1. It was concluded therefore that whole blood samples gave a direct measure of the amount of drug in plasma.

4. BLOOD LEVEL STUDIES

a. Intravenous Dosing

Not less than three male Wistar rats were used in each blood level study. The rats were starved for 24 hours prior to the blood level study and anesthetized with an intraperitional injection of urethane (1.25 g/kg). The femoral vein, femoral artery and the trachea were cannulated using Intrademic[R] polyethylene tubing[a] PE-50 or PE-240 respectively. A blank blood sample (0.4 ml) was collected from femoral artery into heparinized microsample disposable centrifuge tubes[b] at zero time (just prior to administration of the dose). Solutions of ethosuximide, methsuximide and phensuximide were prepared separately in dextrose/saline injection USP. The strength of the solutions prepared was as follows: ethosuximide: 3 mg/ml, 5 mg/ml and 40 mg/ml; methsuximide: 2.8 mg/ml and phensuximide: 4.2 mg/ml. One ml of a solution of ethosuximide or 2 ml of a solution of methsuximide or phensuximide was administered into the femoral vein of the rats through the venous cannula. The cannula was flushed with physiological saline (0.9%) following the dose. Blood samples (0.4 ml) were collected at various time intervals following the dose from the femoral artery in a similar way to the way the blank blood sample was collected. The contents of the tube were well mixed, stoppered and centrifuged[c] for 25 minutes at speed setting of 90. A 50 mcl sample of plasma was extracted and analyzed for free drug according to the procedure described previously.

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[a]Clay Adams, Becton, Dickson and Company, Parsippany, N.J.

[b]Bel-Art Products, Pequannock, N.J.

[c]Adams Dynack Centrifuge Model CT-1300.

An Oxford sampler ™ Micro Pipetting system[a] with disposable tips was used for accurate and quick measureing of plasma samples for analysis.

Following the intravenous (I.V.) injection of ethosuximide to rats at dose levels of 40 mg, 10 mg, 5 mg, and 3 mg, the blood level declined very rapidly within 20 minutes and thereafter the fall in blood level was very slow over a 12 hour period following the injection. Table A-1 (Appendix A) presents the plasma level data for rats 1 - 3 over the period of 12 hours at the 40 mg dose level. Table A-2 and A-3 summarize the plasma level data for six rats over the period of 120 minutes following administration of the 40 mg dose of ethosuximide to rats 4 - 9. Table A-4 lists the plasma level data following 10 mg I.V. dose to rats 10 - 13, and the plasma level data following administration of 5 mg I.V. dose of ethosuximide to rats 14 - 21 are presented in Table A-5. Table A-6 shows the plasma level data following 3 mg I.V. dose of ethosuximide to rats 22 - 25. Figure 13 shows typical plasma level/time profiles for ethosuximide at four dose levels. The zero time plasma concentration of ethosuximide, computed according to a two compartment open model, (discussed later, see page 112), showed a linear relationship with dose, indicating the absence of dose dependent kinetics for ethosuximide within the dose range studied. Figure 14 shows the linear relationship between the zero time plasma concentration and intravenous dose. Wide inter-subject variations in plasma half life of ethosuximide in rats following I.V. dose was observed as shown in Figure 15; however the plasma half life of ethosuximide in rats generally ranged between 12 - 18 hours.

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[a]Oxford Laboratories, Fostercity, California.

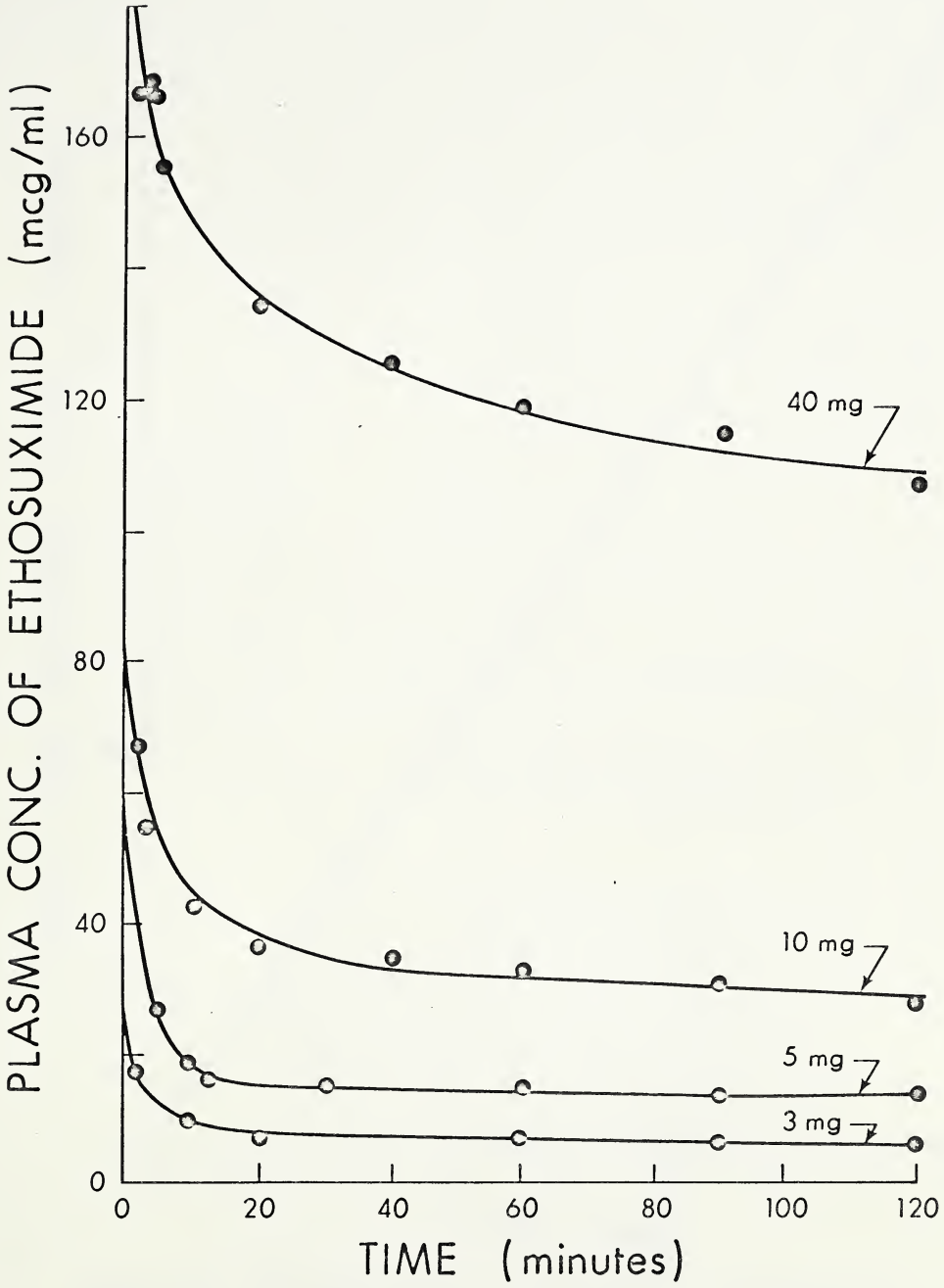

Figure 13. Plasma concentration-time profile of ethosuximide following I.V. administration at various dose levels. Rat 1 (40mg), Rat 10 (10 mg), Rat 14 (5 mg), and Rat 22 (3 mg).

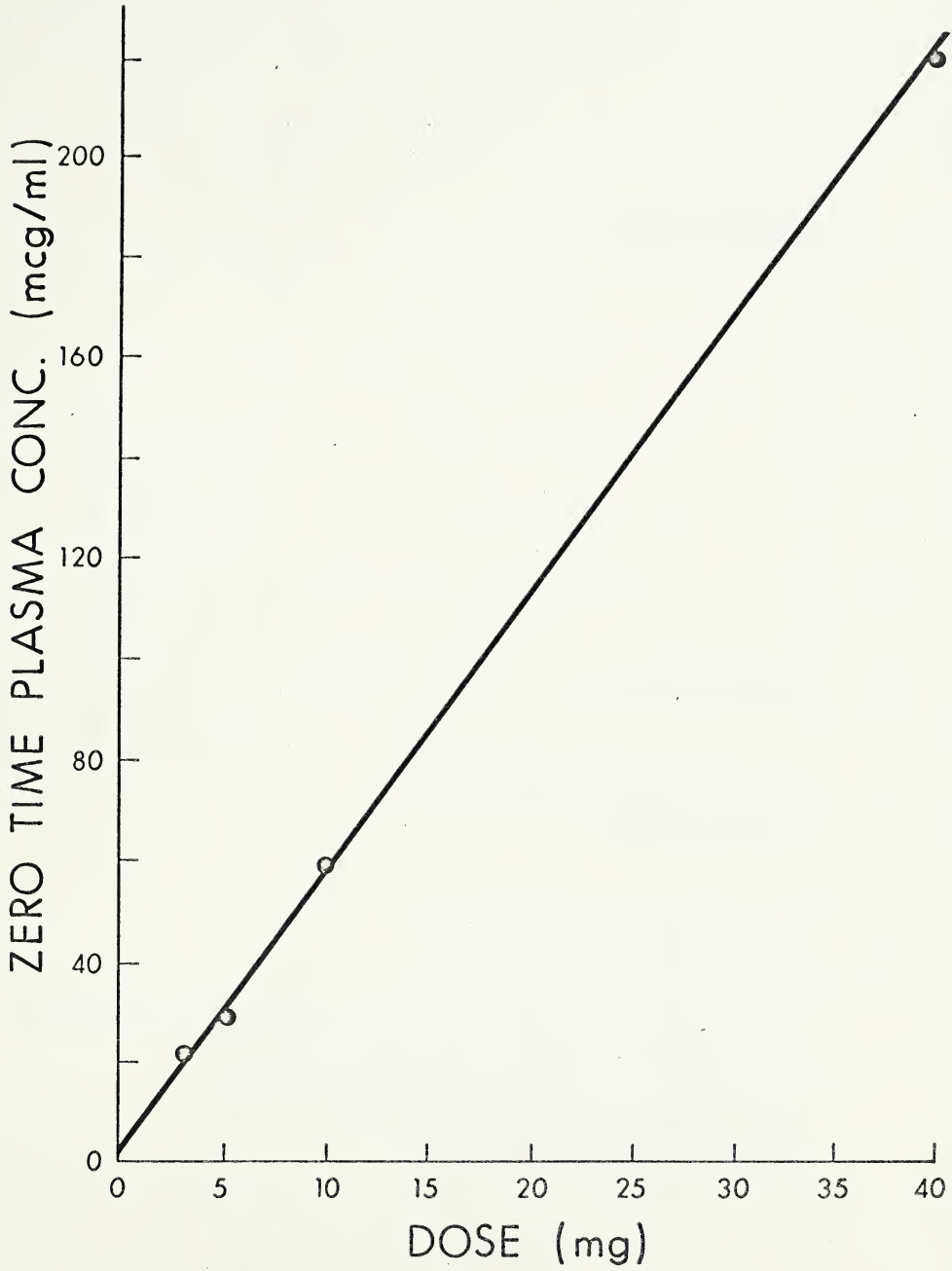

Figure 14. Zero time plasma concentration as a function of dose for ethosuximide following I.V. administration to rats. Each point represents the mean of data obtained from at least three animals.

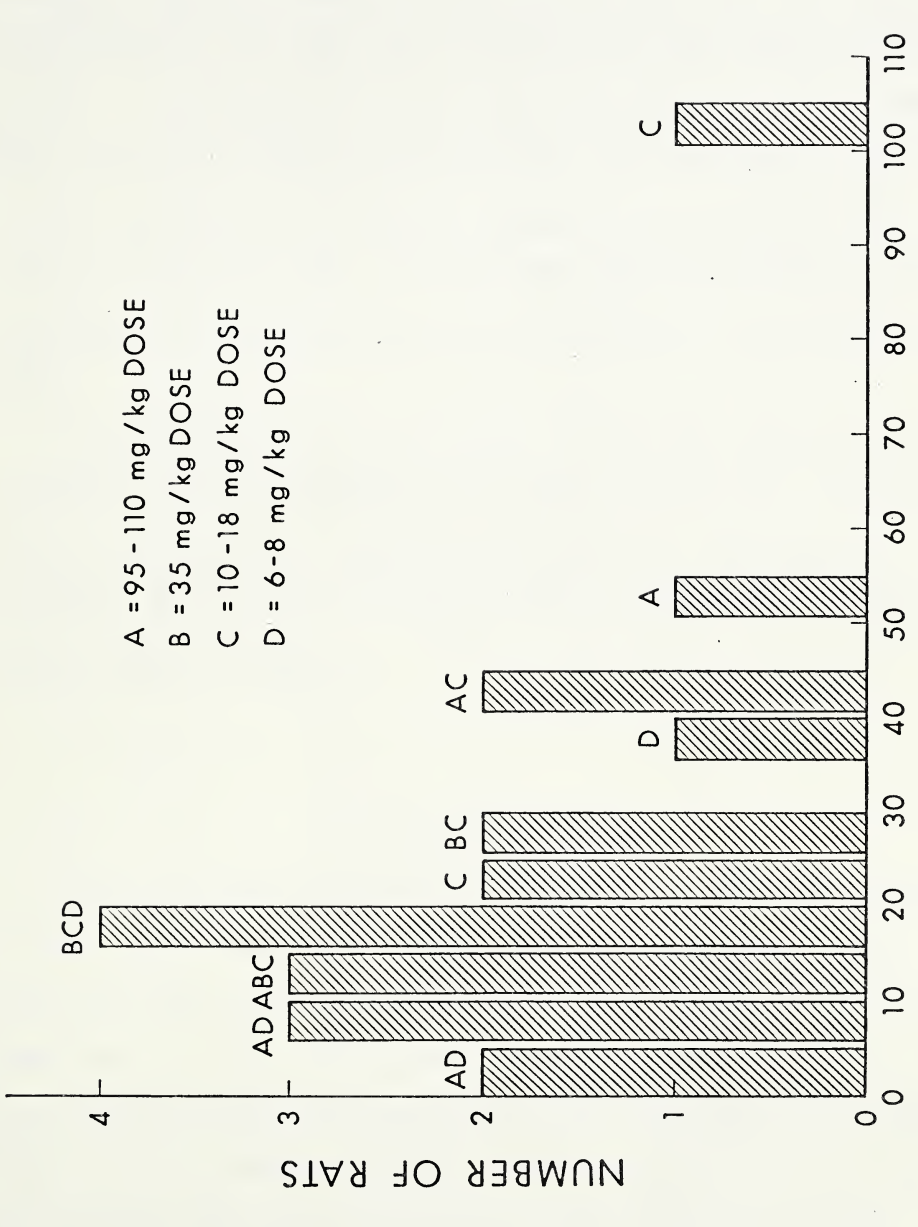

Figure 15. Distribution of biological half life of ethosuximide following I.V. administration in rats. (From I.V. Blood of the drug in rats.)

Plasma level data following intravenous dose of methsuximide to rats 26 - 28 are presented in Table A-7. Table A-8 shows the plasma level data following intravenous dose of phensuximide to rats 29 - 31; a relatively fast decline in the plasma level of methsuximide and phensuximide was noticed. A semilogarithmic plot of the plasma concentration of ethosuximide (Figure 16), methsuximide (Figure 17) and phensuximide (Figure 18) versus time, showed a biexponential decay of the drug plasma level, following an intravenous dose, indicating the need of a minumum of a two compartment pharmacokinetic model for the analysis of the plasma level data.

Some pharmacokinetic rate constants and derived pharmacokinetic parameters obtained from blood level studies following the intravenous dose of ethosuximide are summarized in Table XIV. Pharmacokinetic parameters obtained from intravenous dose of methsuximide and phensuximide are shown in Table XV.

b. Single Oral Dosing

Three male Wistar rats weighing between 300 - 400 g were starved for 24 hours prior to the study. One ml of a solution of ethosuximide (40 mg/ml) was administered orally to each rat by means of an oral dosing needle and a syringe. Prior to and following the oral dose of the drug, a 50 mcl blood sample was collected from tail vein using 50 mcl heparinized disposable pipettes. The blood samples were collected at 5, 15, and 30 minutes and at 1, 2, 4, 8, and 12 hours following the dose. The amount of unchanged drug present in the blood samples was determined as described previously.

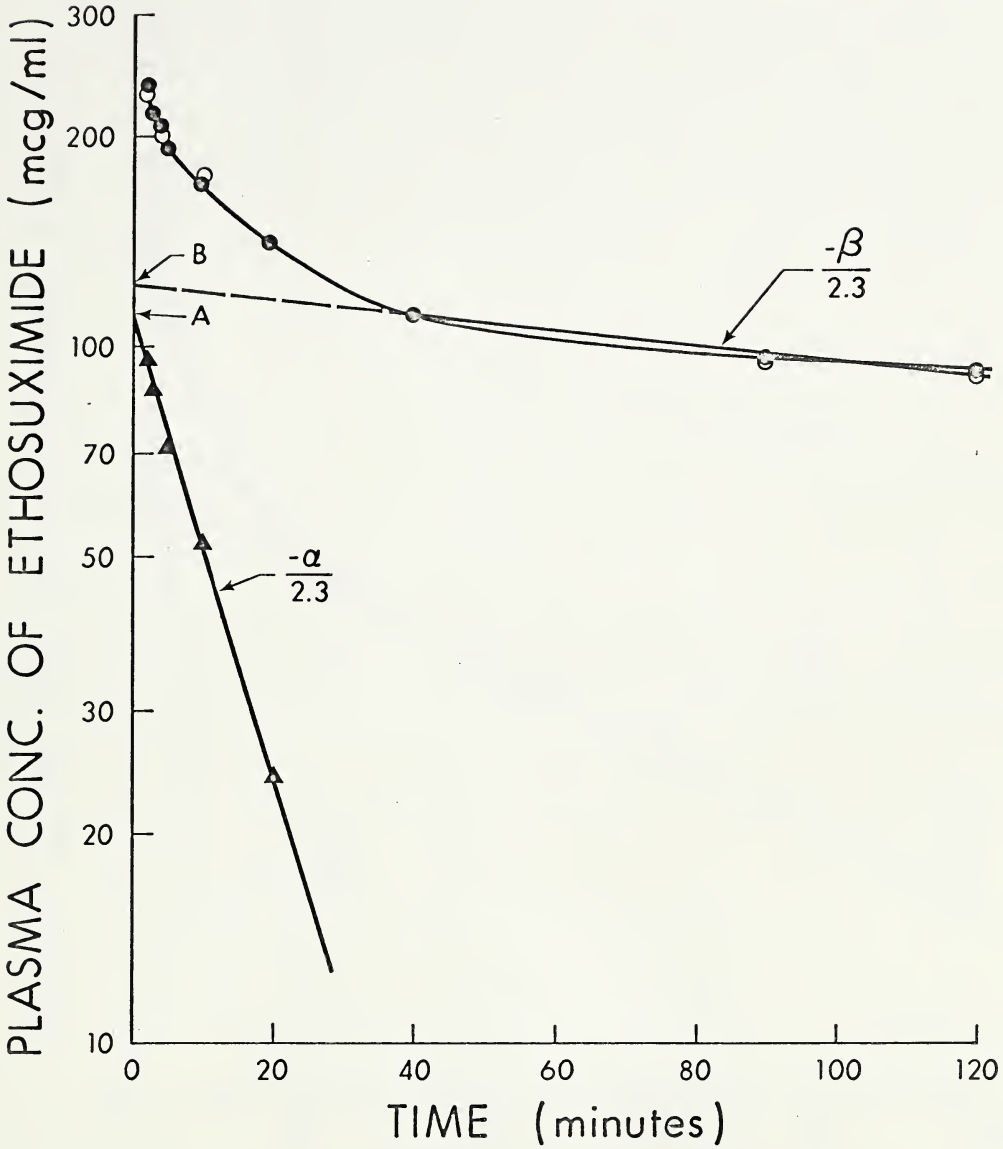

Figure 16. Plasma concentration (mcg/ml) of ethosuximide (●) as a function of time following intravenous administration of 40 mg solution dose. (Rat - 3). (▲) Fast phase residuals. (o) Computer predicted points. A = 130 mcg/ml, B = 98.8 mcg/ml, α = 0.0567/min, β = 0.00055/min.

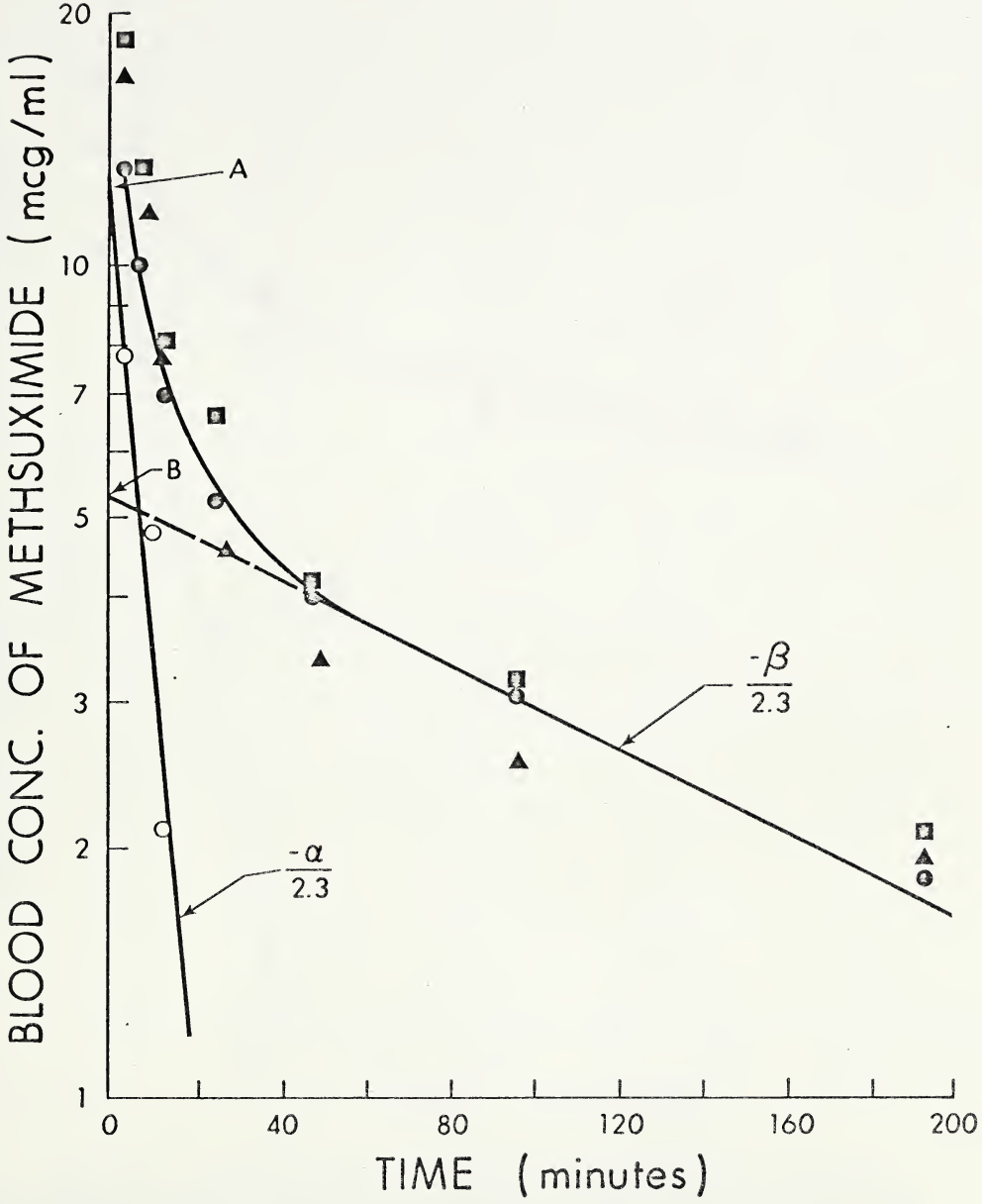

Figure 17. Blood level-time profile of methsuximide following intravenous dose of 5.6 mg to rat 26 (●), 27 (■) and 28 (▲). (o) Fast disposition phase residuals. A = 16.7 ± 2.2 mcg/ml, B = 4.98 ±0.28 mcg/ml, α = 0.14 ± .0008 min^{-1}, and β = 0.00429 ± 0.00099 min^{-1}.

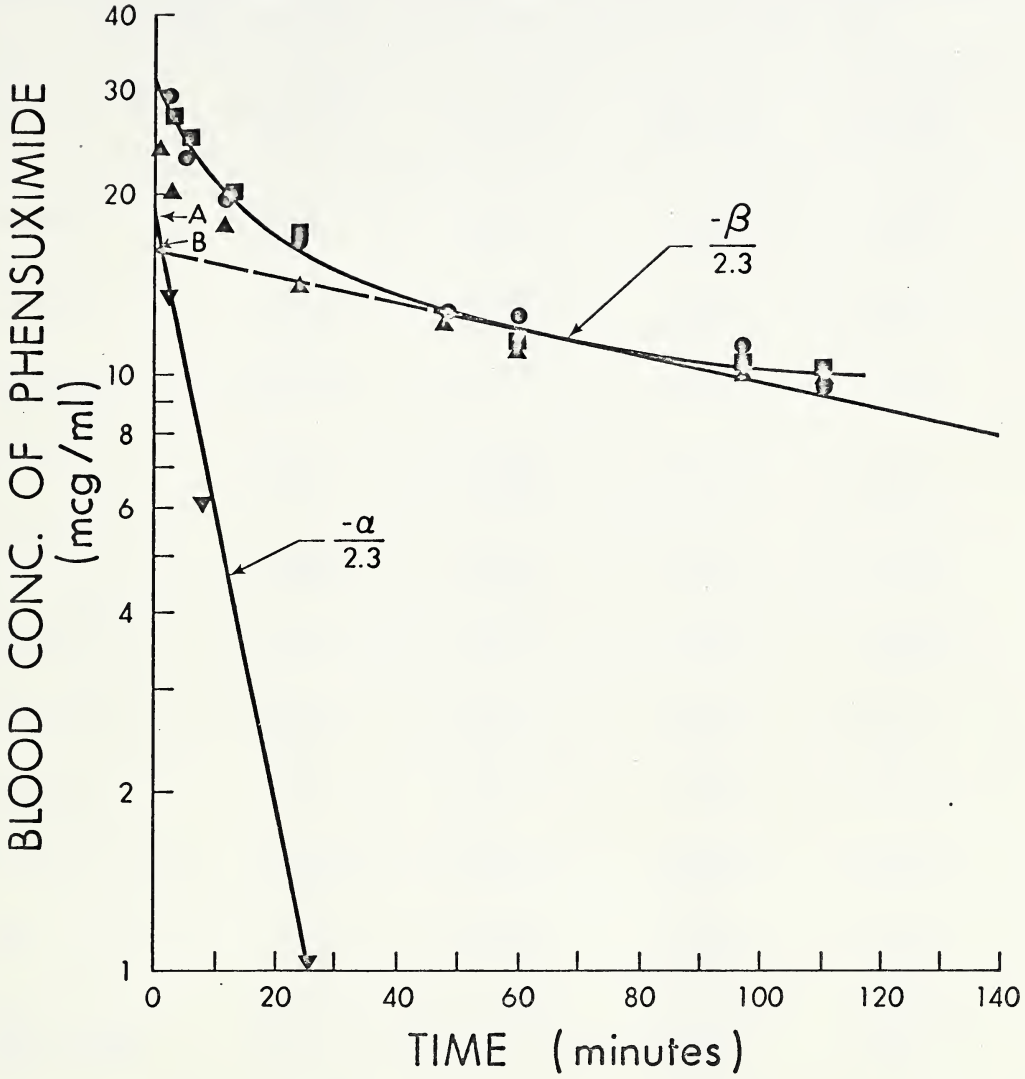

Figure 18. Blood level-time profile of phensuximide following intravenous administration of 8.4 mg dose to rats, 29 (●), 30 (■) and 31 (▲). (▼) Fast disposition phase residuals. A = 13.7 ± 2.8 mcg/ml, B = 17.1 ± 0.5 mcg/ml, α = 0.131 ± 0.016 min^{-1}, β = 0.00535 ± .0001 min^{-1}.

TABLE XIV

Pharmacokinetic parameters of ethosuximide according to a two compartment open model, following intravenous injection to Wistar rats.

Parameters[b]	40 mg Dose N = 6	10 mg Dose N = 4	5 mg Dose N = 8	3 mg Dose N = 4
A mcg/ml	94.27 ± 42[a]	21.31 ± 9.80	15.85 ± 8.30	13.04 ± 1.10
B mcg/ml	121.03 ± 21.60	37.96 ± 2.60	14.56 ± 5.12	8.94 ± 2.39
C^0 mcg/ml (A + B)	215.30	59.27	30.41	21.98
α hr^{-1}	5.77 ± 2.22	7.392 ± 1.830	5.57 ± 2.28	13.8 ± 2.50
β hr^{-1}	0.075 ± 0.068	0.041 ± 0.014	0.032 ± 0.016	0.109 ± 0.066
Kct hr^{-1}	2.128 ± 0.41	3.43 ± 1.01	3.43 ± 1.18	7.970 ± 0.591
Ktc hr^{-1}	3.60 ± 2.32	3.53 ± 1.35	3.63 ± 1.15	5.72 ± 1.90
Ktc/Kct	1.69	1.03	1.050	0.711
K hr^{-1}	0.119 ± 0.085	0.061 ± 0.012	0.064 ± 0.034	0.241 ± 0.259
β/K	0.589 ± 0.128	0.659 ± 0.118	0.510 ± 0.090	0.406 ± 0.058
Vc % Body Weight	48.21 ± 8.3	60.50 ± 8.10	47.42 ± 9.30	32.20 ± 4.20
Vt % Body Weight	34.17 ± 12.97	32.42 ± 12.90	45.31 ± 11.10	48.08 ± 14.15
Vc/(Vc + Vt)	0.593	0.655	0.513	0.410
A/α	18.84	4.020	1.99	0.959
$T_{1/2}$ (hr)	21.27 ± 20.86	18.44 ± 6.70	33.02 ± 30.50	17.27 ± 16.49

[a]Standard deviation.

[b]Definition of the symbols see Appendix B.

TABLE XV

Pharmacokinetic parameters of methsuximide and phensuximide according to two compartment open model, following intravenous injection to Wistar rats.

Parameters[b]	Methsuximide N = 3 Dose = 5.6 mg	Phensuximide N = 3 Dose = 8.4 mg
A mcg/ml	16.712 ± 2.24[a]	13.73 ± 2.84
B mcg/ml	4.980 ± 0.28	17.13 ± 0.50
C^0 mcg/ml (A + B)	21.69	30.86
α hr^{-1}	8.40 ± 0.08	7.86 ± 1.63
β hr^{-1}	0.257 ± 0.093	0.321 ± 0.034
Kct hr^{-1}	5.15 ± 0.59	2.98 ± 0.46
Ktc hr^{-1}	2.2 ± 0.049	4.65 ± 1.51
Ktc/Kct	0.427	1.56
K hr^{-1}	0.982 ± 0.25	0.554 ± 0.052
β/K	0.262 ± 0.06	0.579 ± 0.13
Vc % Body Weight	65.77 ± 12.3	67.91 ± 3.12
Vt % Body Weight	168.8 ± 27.3	46.57 ± 16.6
Vc/(Vc + Vt)	0.280	0.593
A/α	1.98	1.74
$T_{1/2}$ hr	3.02 ± 1.33	2.18 ± 0.266

[a]Standard deviation.

[b]Definition of symbols see Appendix B.

A semi-logarithmic plot of blood level-time profile for ethosuximide following oral administration of 40 mg dose in solution to a rat is shown in Figure 19. The elimination rate constant K was determined from the slope of the linear portion of the tail end of plasma level-time curve. The points were chosen sufficiently beyond the time during which absorption or distribution phenomena might cause deviation from linearity. The ascending portion of the curve was subjected to a feathering technique. The slope of the feathered line gave the absorption rate constant Ka. The fraction of the dose absorbed FD/V was obtained from the intercept at the Y-axis by dividing the intercept value by Ka and multiplying the quotient by (Ka-K). Symbol A_o has been used in Figure 19 to denote FD/V. Plasma level data from rats 32 - 34 following an oral dose of ethosuximide in solution are presented in Table A-9. Graphical estimation of area under the plasma level-time curve is illustrated in Figure 20. The peak plasma level was reached within three hours following the oral dose. Best estimates of pharmacokinetic rate constants and some derived pharmacokinetic parameters according to a 'two' compartment open model analysis of the plasma level data is presented in Table XVI.

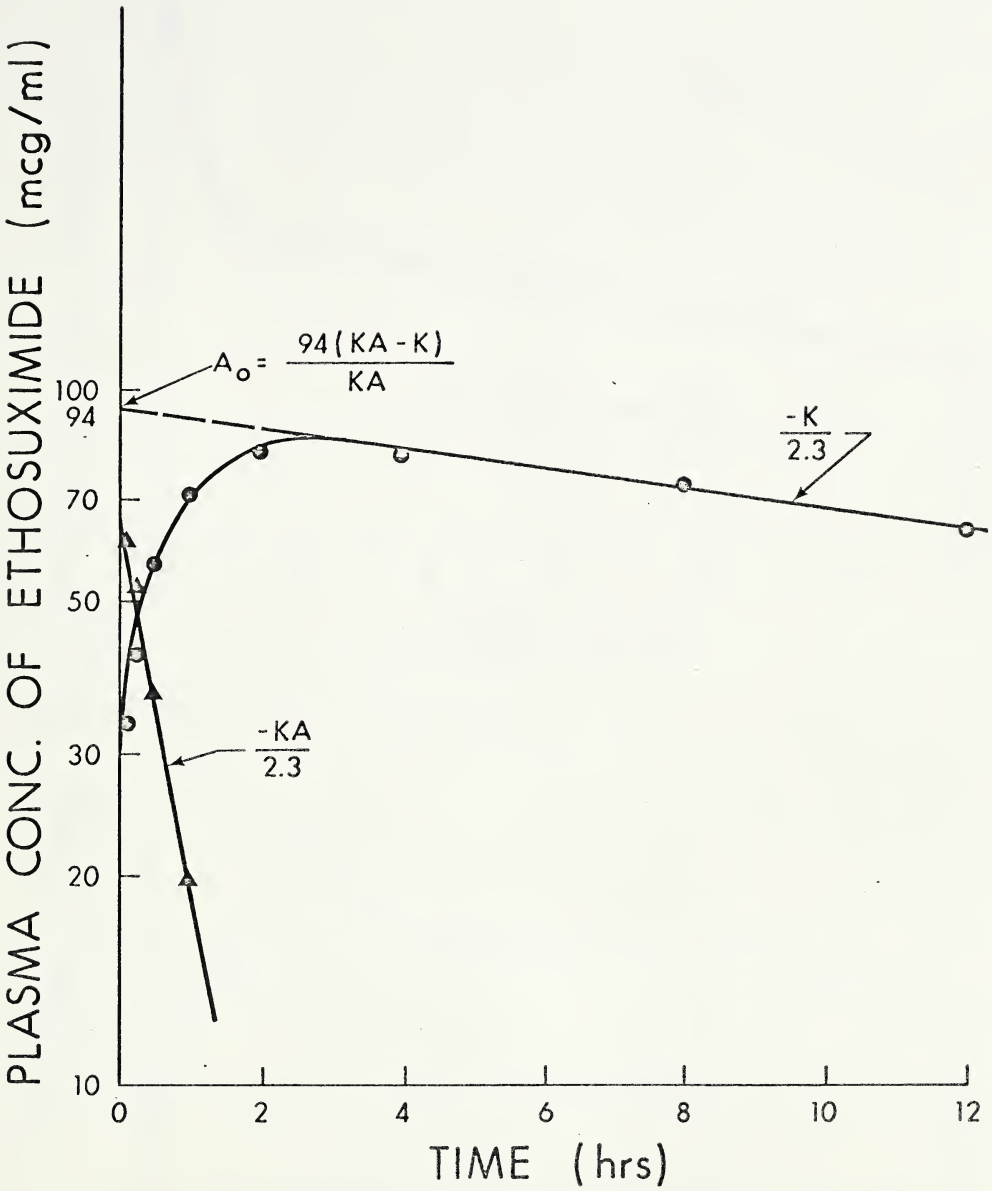

Figure 19. Plasma concentration of ethosuximide (mcg/ml) (●) as a function of time following 40 mg oral solution dose to Rat 33. (▲) Feathered points.

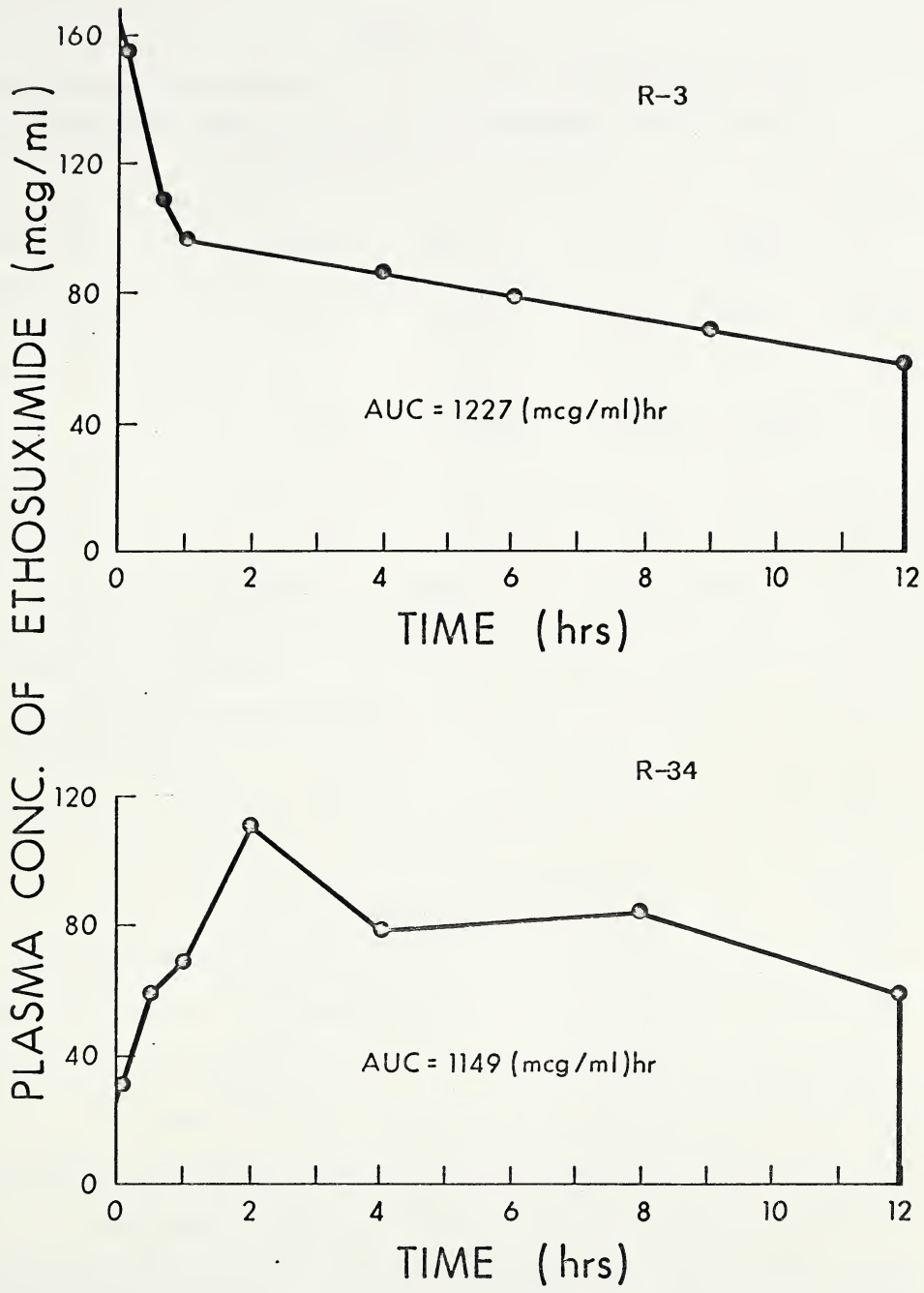

Figure 20. Typical plasma concentration-time profile of ethosuximide (40 mg dose) following I.V. dose to Rat - 3 and oral dose to Rat - 34. Area under the curve (AUC) was determined by counting the squares.

TABLE XVI

Pharmacokinetic parameters of ethosuximide according to a 'two' compartment open model, following administration of 40 mg dose of ethosuximide in solution.

Parameters	Rat 28	Rat 29	Rat 30	Mean
FD/V (mcg/ml)	165.73	81.67	101.97	116.45 ± 43.8[a]
Ka (hr^{-1})	1.04	2.89	2.01	1.97 ± 0.93
K (hr^{-1})	0.086	0.016*	0.038	0.047 ± 0.03
$T_{1/2}$ (hr)	8.1	37.6*	18.42	21.37 ± 8.6
T_{max} (hr)	2.62	1.75	2.02	2.13 ± 0.44
AUC[b] (mcg.hr/ml)	1484.0	1088.0	1149.0	1240.0 ± 213

[a]Standard deviation.

[b]To 12 hours following dose.
*Not reliable.

c. Multiple Oral Dosing

Three rats were given 40 mg of ethosuximide every 12 hours orally as described above. Blood samples (50 mcl) were collected as described above just prior to administration of doses at 12, 24, 36. 48, 60, 72, 84, 96 and 108 hours following the first dose. Following the last dose, at 108 hours, blood samples were collected at 112, 122 and 145 hours to check for any change in the overall elimination rate constant during the multiple dosing regimen. The unchanged ethosuximide in the blood samples was determined in the usual manner.

Rats 32 - 34 received 40 mg dose of ethosuximide every twelve hours following the first dose. The blood concentration of ethosuximide found

just prior to subsequent doses is presented in Table A-10. Figure 21 shows the relationship between the computer predicted points and the observed points. A two compartment model seemed to predict plasma concentration values fairly close to observed values. Table XVII lists the plasma concentration following multiple doses in Rat 34, together with predicted values according to one or two compartment models.

TABLE XVII

Blood concentration of ethosuximide following a multiple dose regimen (40 mg every 12 hours) in Rat 34

Time[a]	A (mcg/ml)	B (mcg/ml)	C (mcg/ml)	D (mcg/ml)
12.0	61.85	66.18	66.0	52.9
24.0	66.14	108.3	104.6	74.74
36.0	121.3	135.2	127.1	83.37
48.0	66.02	152.3	140.3	87.43
60.0	67.05	163.2	147.9	88.96
72.0	56.22	170.1	152.4	89.59
84.0	72.6	174.5	155.0	89.85
96.0	62.6	177.2	156.5	89.95
108.0	87.5	179.1	157.4	90.0

[a]Time following the initial dose, A = observed blood concentration of ethosuximide, B = predicted concentration of ethosuximide according to one compartment model, C = predicted blood concentration of ethosuximide according to a two compartment model assuming K = β (Elimination rate constant obtained from the slope of slow disposition phase), D = predicted blood concentration of ethosuximide according to a two compartment model ($K = \alpha\beta/Ktc$), Ka = 2.0066/hr, K = 0.1185/hr, Kct = 2.13/hr, Ktc = 3.6/hr, β = 0.0376/hr, Vc = 0.2 L, $C_{max\ 1}$ = 237 - 262 mcg/ml, $C_{max\ 2}$ = 186 - 192 mcg/ml, where max_1 and max_2 refer to one and two compartment model respectively.

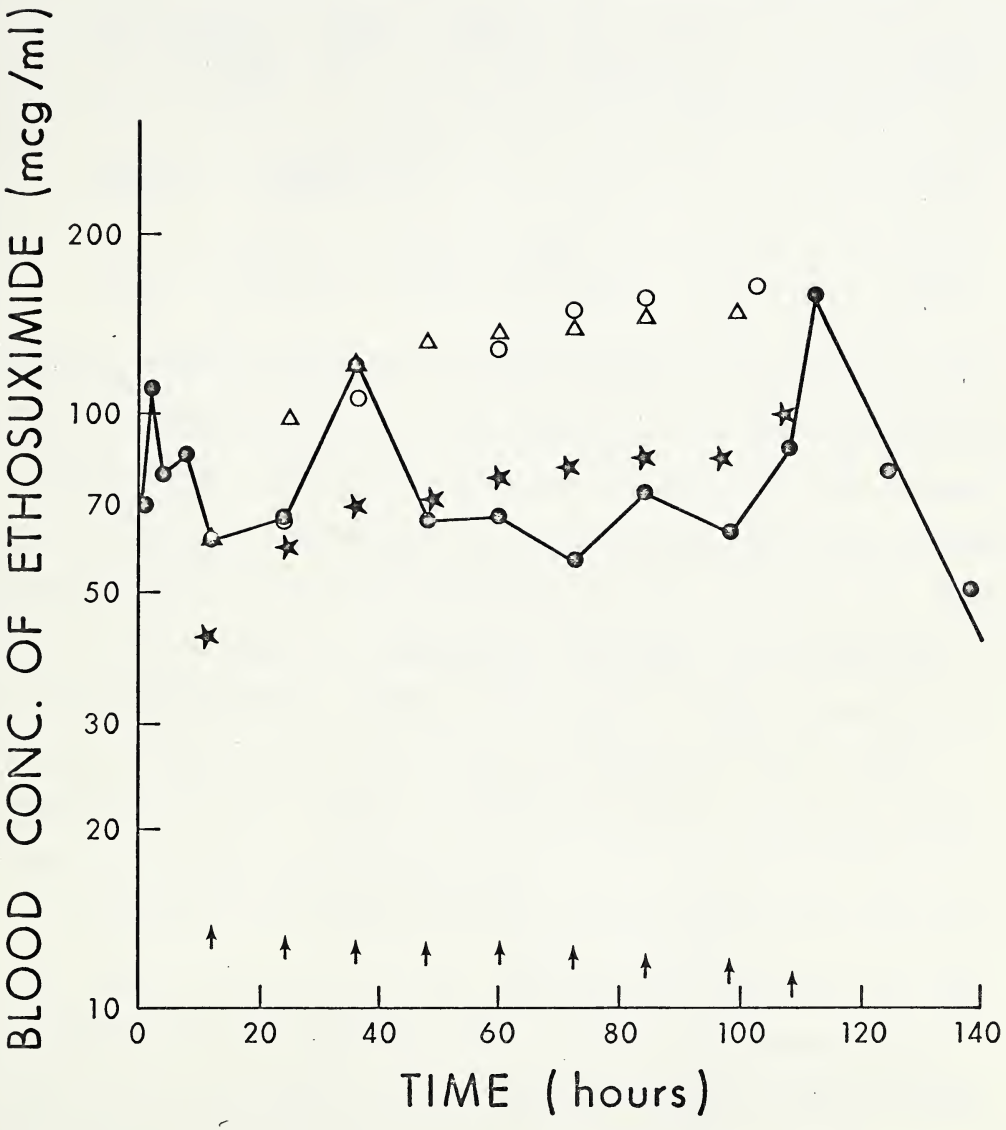

Figure 21. Blood level of ethosuximide following oral multiple dose regimen (40 mg every 12 hours) to Rat - 34, as a function of time. (↑) Point of dosing and blood sampling, (●) Observed points, (o) Computer predicted points according to a 'two' compartment model, (Δ) Computer predicted point according to a two compartment model shere $K = \beta$, (*) Computer predicted points according to a two compartment model where $K = \alpha\beta/Ktc$.

The equations 34 - 36 were used to predict blood levels during the multiple dose regimens according to a one compartment open model.

$$Ct = \frac{Ka(A_o)_n}{(Ka-K)} (e^{-Kt} - e^{-Kat}) + (C_o)_n e^{-Kt} \quad . \quad . \quad . \quad (34)$$

$$(Dg/V)_n = (FD/V) e^{-Kat} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (35)$$

$$(A_o)_n = (FD/V)_n + ((FD/V) e^{-Ka\tau})_{n-1} \quad . \quad . \quad . \quad . \quad (36)$$

where A_o = FD/V, the fraction of dose available at the absorption site which is absorbed per volume of distribution. C_t is the concentration of ethosuximide in the blood at time t, Ka is absorption rate constant, K is elimination rate constant, C_o is the concentration of ethosuximide in blood at the start of the nth dosing interval, τ is the dosing interval, Dg is the amount of ethosuximide in the gut, V is the apparent volume of distribution, $(FD/V)_n$ is the fraction of dose absorbed per volume of distribution from nth dose and $((FD/V) e^{-Ka\tau})_{n-1}$ is the fraction of dose per volume of distribution which is remaining in the gut from previous dose for absorption.

Equations used for the prediction of blood levels during multiple dose regimen according to a two compartment open model were the same as those reported by Niebergall et al. (1974). The computer program reported by these authors was modified and rewritten in FOCAL language to suit our Digital PDP-8/L[a] computer and the predicted values reported above for one and two compartment open models were calculated. Figure 22 shows the predicted and observed plasma levels of ethosuximide in man according to one and two compartment open models. The observed

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[a]Digital Equipment Corporation, Maynard, Massachusetts.

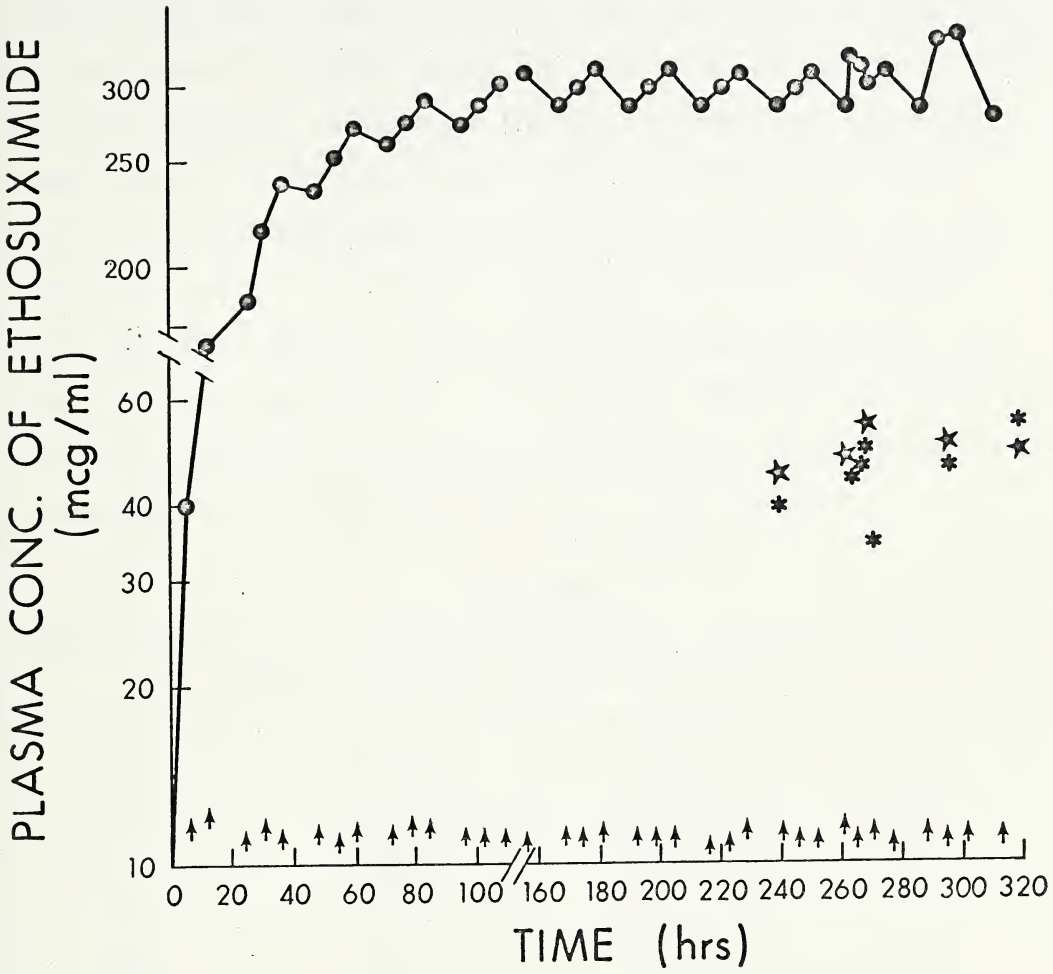

Figure 22. Plasma concentration of ethosuximide following multiple oral dose regimen (250 mg t.i.d.) of Zarontin syrup administered at 8:00 AM, 2:00 PM and 6:00 PM to subject 1 (Hearer et al., 1970). (●) Computer predicted points according to one compartment open model (Ka = 2.85/hr, K = 0.0269/hr, FD/V = 46.5 mcg/ml, from Buchanan et al., 1969). (*) Observed points. (★) Predicted points according to two compartment open model. (Ka = 2.85/hr, K = 0.03/hr, Kct = 2.13/hr, Ktc = 3.6/hr and Vc = 5.0 L)

plasma levels were those reported by Hearer et al. (1970) for subject 1 following multiple oral dosing with Zarontin[R] (ethosuximide) syrup, dose 250 mg three times a day. The value of FD/V, the fraction of dose absorbed per volume of distribution, was that reported by Buchanan et al. (1969) for their subject 1. Predictions from the two compartment open model were found to fit the observed data. The rate constants Kct and Ktc used in the calculation were the same as those found in rats since the published reports did not contain sufficient information to determine the needed rate constants. It was assumed that the rate constants Kct and Ktc were not much different in man and rat. Observed and predicted values for the plasma concentration of ethosuximide, in rats 32 and 33 are presented in Table XVIII and XIX, and in man in Table XX.

TABLE XVIII

Blood concentration of ethosuximide following a multiple oral dose regimen (40 mg every 12 hours) to rat 32.

Time[a] (hr)	A (mcg/ml)	B (mcg/ml)	C (mcg/ml)
12.0	59.44	106.7	54.88
24.0	96.04	172.7	77.50
36.0	109.88	213.5	86.80
48.0	97.60	238.8	90.66
60.0	----	254.4	92.24
72.0	71.99	264.1	92.89
84.0	66.90	270.1	93.16
96.0	81.99	271.8	93.27
108.0	98.68	276.1	93.32

[a]Time following the initial dose, A = observed blood concentration of ethosuximide, B = predicted blood concentration of ethosuximide according to one compartment open model, C = predicted blood concentration of ethosuximide according to two compartment open model, K = 0.1185/hr, Ka = 1.0387/hr, Kct = 2.13/hr, Ktc = 3.6/hr, β = 0.040/hr, Vc = 0.2L, $C_{max\ 1}$ = 373 - 393 mcg/ml, $C_{max\ 2}$ = 178 - 180 mcg/ml, ----sample lost.

TABLE XIX

Blood concentration of ethosuximide following multiple oral dose regimen (40 mg every 12 hours) to rat 33.

Time[a] (hr)	A (mcg/ml)	B (mcg/ml)	C (mcg/ml)
12.0	62.59	51.20	52.32
24.0	52.40	82.90	73.88
36.0	45.50	102.60	82.76
48.0	70.80	114.70	86.42
60.0	114.20	122.10	87.93
72.0	81.50	126.80	88.55
84.0	79.60	129.80	88.81
96.0	75.50	131.50	88.91
108.0	75.90	132.60	88.96

[a]Time following the initial dose, A = observed blood concentration of ethosuximide, B = predicted blood concentration of ethosuximide according to a 'two' compartment open model, C = predicted blood concentration of ethosuximide according to a two compartment open model, Ka = 2.89/hr, K = 0.1185/hr, Kct = 2.13/hr, Ktc = 3.6/hr, β = 0.04, Vc = 0.2 L, $C_{max\ 1}$ = 197 - 202 mcg/ml, $C_{max\ 2}$ = 195 - 197 mcg/ml.

TABLE XX

Observed and predicted plasma concentration of ethosuximide in man following oral multiple dosing (250 mg t.i.d.). Data obtained from Haerer et al. (1970) and Buchnan et al. (1969) subject 1.

Time[a] (hr)	A (mcg/ml)	B (mcg/ml)	C (mcg/ml)
6.0	-	39.9	5.23
12.0	-	73.9	9.9
24.0	-	127.5	17.8
48.0	-	1198.3	29.1
84.0	-	240.0	38.93
102.0	-	250.8	41.84
180.0	-	165.9	47.37
204.0	-	266.9	47.98
240.0	39.0	267.6	48.49
261.0	-	290.3	51.52
264.0	44.2	267.8	48.69
266.0	45.0	-	-
267.0	-	290.3	51.5
268.0	48.8	-	-
270.0	34.0	267.8	48.7
294.0	46.2	267.9	48.8
318.0	53.8	268.0	48.9
357.0	-	290.5	51.82
384.0	-	268.0	49.0

[a]Time following the initial dose. A = observed blood concentration of ethosuximide in subject 1 (Haerer et al., 1970), B = predicted blood concentration of ethosuximide according to a 'two' compartment open model, where Ka = 2.85/hr and K = 0.0269/hr, and FD/V = 46.5 mcg/ml (Buchanan et al., 1969), C = predicted blood level of ethosuximide according to two compartment open model, where Ka = 2.85/hr, K = $\alpha\beta$/Ktc = 0.03/hr, Kct = 2.13/hr, Ktc = 3.6/hr, β = 0.02/hr and V_c = 5.0 L, C_{max} in a 'two' compartment at equilibrium was 340 - 378 mcg/ml, C_{max} in two compartment model at equilibrium was 53.7 mcg/ml.

5. IN-VIVO AVAILABILITY STUDY

All chemical and adjuvants used in this study were of USP grade. Dosage forms, other than commercial preparations, were prepared by vigorously shaking weighed quantities of ethosuximide with the respective adjuvant or antacid in a 25 ml screw capped vial with a suitable vehicle. All dosage forms were prepared immediately before use. The details of the dosage forms, the adjuvants and the vehicles used are described below.

a) Ethosuximide solution 40 mg/ml in dextrose/saline injection USP. Dose = 1 ml, intravenously.

b) Zarontin^{R} Syrup, Park - Davis and Company (250 mg/5ml) lot DB115 purchased from Belvedere Drugs, Edmonton, Alberta. Dose = 0.8 ml, orally.

c) Ethosuximide solution 40 mg/ml in dextrose/saline injection USP to be given orally. Dose = 1 ml, orally.

d) Zarontin^{R} Capsule, Park - Davis and Company (250 mg/capsule) contents of two capsules was diluted with PEG-400 to make 10 ml. Lot DA127 purchased from supplier listed under b. Dose = 0.8 ml orally.

e) Ethosuximide 400 mg dissolved in 1.5% methycellulose (in dextrose/saline injection USP) to make 10 ml. Dose = 1 ml orally.

f) One g activated charcoal and 400 mg ethosuximide suspended in dextrose/saline injection USP to make 10 ml. Dose = 1 ml orally.

g) Ethosuximide 400 mg dissolved in olive oil to make 10 ml. Dose = 1 ml orally.

h) One g of calcium phosphate tribasic and 400 mg of ethosuximide suspended in dextrose/saline injection USP to make 10 ml. Dose = 1 ml orally.

i) One g calcium chloride and 400 mg ethosuximide dissolved in dextrose/saline injection USP to make 10 ml. Dose = 1 ml orally.

j) One g of calcium carbonate and 400 mg of ethosuximide suspended in dextrose/saline injection to make 10 ml. Dose = 1 ml orally.

k) One g dried aluminum hydroxide gel and 400 mg of ethosuximide in dextrose/saline injection USP to make 10 ml. Dose = 1 ml orally.

l) One g magnesium trisilicate and 400 mg ethosuximide suspended in dextrose/saline injection USP to make 10 ml. Dose = 1 ml orally.

Protocol. Thirty-six male Wistar rats weighing between 300 and 500 g were used in this study. The rats were randomly assigned to twelve treatment groups of three rats each. A completely random design (Steel and Torrie, 1960) was used in this investigation. The rats were fasted for 24 hours prior to treatment but water was made available for drinking. On the day of the experiment the rats in a particular treatment group received their treatment by the appropriate route. Fifty mcl blood samples were collected from a tail vein at 0, 0.5, 1.0, 2.0, 3.0, 6.0, 9.0, and 12.0 hours following the dose. The concentration of

ethosuximide in the blood samples taken at the various times was determined according to the procedure described earlier.

The blood levels of ethosuximide in rats after a number of different treatments is presented in Table A-1, Table A-9 and Tables A-11 to A-20. The area under the blood concentration-time curve for each animal was determined by counting the squares under the curve as illustrated in Figure 20 for an I.V. and an oral dose of ethosuximide solution. The graphical estimates of the absorption rate constant, Ka, the elimination rate constant, K, and the fraction of dose absorbed per volume of distribution, FD/V, from the blood data of each animal were determined as illustrated in Figure 19 and described earlier under the blood level study. The best estimates of various pharmacokinetic parameters determined by digital computer least-square iteration using program 'NONLIN' are summarized in Table XXI.

A typical plasma level-time curve following treatment with the various formulations studied are illustrated in Figure 23. The influence of various formulations and adjuvants on the systemic availability of ethosuximide is shown in Figure 24. The area under the plasma level-time curve for the intravenous dose was used as a reference for the computation of the percent systemic availability of ethosuximide in rats over 12 hour period.

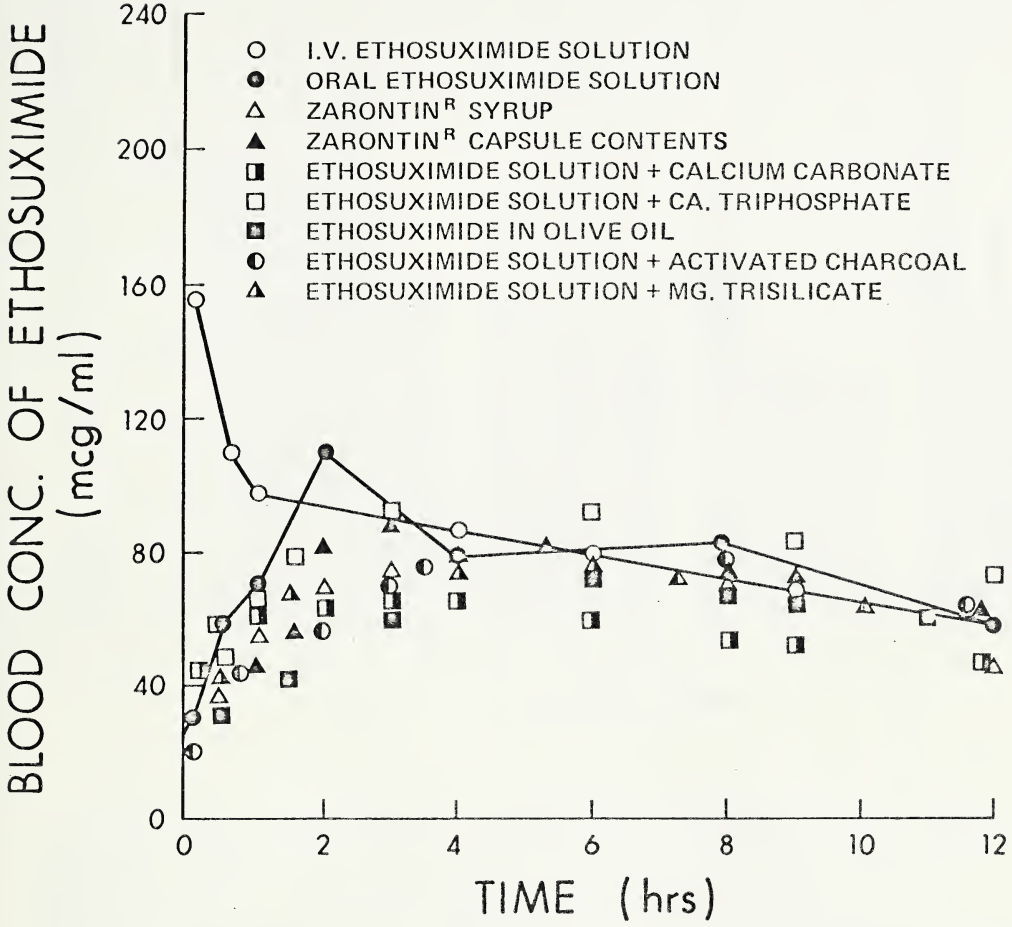

Figure 23 Typical plasma level-time curve for various formulations of ethosuximide in male Wistar rats. (o) Rat 3; (●) Rat 33; (△) Rat 34; (▲) Rat 39; (▣) Rat 55; (□) Rat 51; (▪) Rat 48; (◐) Rat 43; (◭) Rat 63.

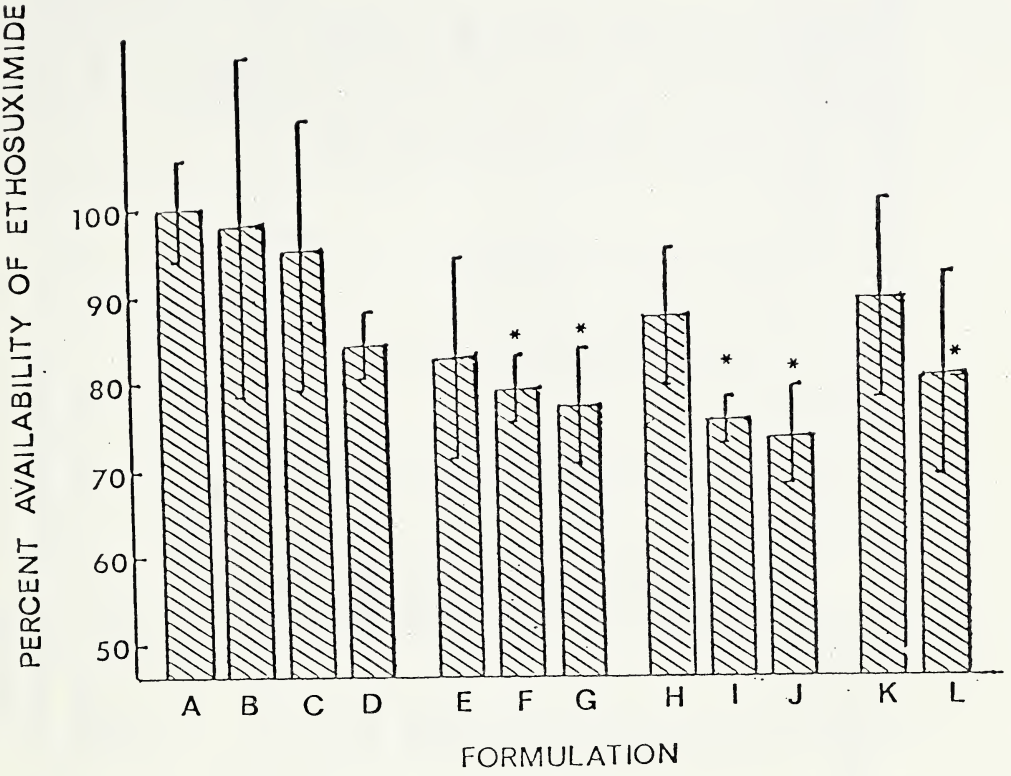

Figure 24. Percent systemic availability of ethosuximide from different formulations over the 12 hours following dosing. A = ethosuximide solution I.V.; B = Zarontin syrup oral; C = ethosuximide solution oral; D = Zarontin capsule contents with PEG-400; E = C + 1.5% methylcellulose; F = C + activated charcoal; G = ethosuximide in olive oil; H = C + calcium phosphate tribasic; I = C + calcium chloride; J = C + calcium carbonate; K = C + dried aluminum hydroxide gel; L = C + magnesium trisilicate; $\int$ = standard deviation.

*Statistically significant reduction in percent systemic availability.

TABLE XXI

Influence of formulation and formulation variables on the pharmacokinetic parameters of ethosuximide in Wistar rats. (Blood level data analyzed according to two compartment open model using program NONLIN)

Dose = 40 mg

FN	FD/V (mcg/ml)	Ka (hr^{-1})	K (hr^{-1})	AUC[b] (mcg/hr/ml)	$T_{1/2}$ (hr)	T_{max} (hr)	C_{max} (mcg/ml)
A	-	-	0.043 0.01[a]	1298.0 75.9	16.8 3.8	-	-
B	109.5 ± 10.4	1.54 ± 1.1	0.042 ± 0.012	1281.0 ± 259.6	17.4 ± 4.5	2.85 ± 1.08	98.5
C	116.5 ± 43.8	2.97 ± 0.93	0.047 ± 0.034	1240.0 ± 213.0	21.4 ± 11.6	2.13 ± 0.44	106.2
D	106.9 ± 20.4	0.50 ± 0.10	0.055 ± 0.003	1091.0 ± 49.2	12.4 ± 0.6	6.30 ± 3.0	79.8
E	93.3 ± 22.6	1.46 ± 1.16	0.031 ± 0.027	1069.0 ± 162.0	39.8 ± 19.8	3.58 ± 1.62	84.8
* F	85.4 ± 10.0	0.98 ± 0.57	0.02 ± 0.010	1035.0 ± 48.4	47.9 ± 19.5	4.55 ± 1.14	78.6
* G	78.1 ± 4.4	0.81 ± 0.44	0.011 ± 0.010	985.0 ± 88.6	61.8 ± 17.9	6.52 ± 1.90	73.3
H	102.7 ± 6.3	1.48 ± 0.60	0.028 ± 0.008	1295.0 ± 122.0	25.0 ± 6.9	3.40 ± 0.11	94.5
* I	115.3 ± 28.0	0.38 ± 0.29	0.062 ± 0.036	960.0 ± 34.2	16.6 ± 14.6	6.81 ± 1.58	79.9
* J	79.6 ± 19.2	2.05 ± 1.90	0.037 ± 0.030	933.0 ± 77.6	32.9 ± 25.1	2.61 ± 1.20	73.2
K	107.1 ± 13.95	1.03 ± 0.70	0.042 ± 0.004	1164.0 ± 151.0	16.5 ± 1.7	3.22 ± 0.26	93.5
* L	87.6 ± 14.20	1.58 ± 0.17	0.033 ± 0.030	993.0 ± 147.0	28.5 ± 15.3	2.58 ± 0.26	80.6

FN = Formulation, [a]Standard deviation, [b]to 12 hours following dose, A = ethosuximide solution I.V., B = Zarontin syrup oral, C = ethosuximide solution oral, D = Zarontin capsule contents + PEG-400, E = C + 1.5% methylcellulose, F = C + activated charcoal, G = C + olive oil, H = C + calcium phosphate tribasic, I = calcium chloride + C, J = C + calcium carbonate, K = C + dried aluminum hydroxide gel, L = C + magnesium trisilicate.

*Statistically significant difference.

_____ Unreliable.

6. URINARY EXCRETION STUDY

The urinary excretion of ethosuximide in rats was studied under four treatment conditions, uncontrolled, acid, alkaline and probenecid. Experimental details of the study under each treatment are described below.

a) Urinary excretion study of ethosuximide under uncontrolled urinary pH. Three healthy male Wistar rats weighing 540, 486 and 457 g were starved for 24 hours and placed in individual metabolism cages with water but no food. Blank urine samples were collected. The rats were then given 40 mg of ethosuximide solution orally. The urine excreted, along with the washings of the metabolism cages was collected at 12.5, 24.5, 36, 48.5, 60.5, 72.5, 84.5, and 98 hours following the oral dose. The urines were stored at 4^{0}C until analyzed. The volume of the urine together with washings from the cage was accurately measured (range 30 - 100 ml). A 1 ml aliquot of the diluted urine was analyzed for free ethosuximide as described previously. The rats were fed regular diet 12 hours following the oral dose.

b) Urinary excretion study of ethosuximide under controlled acid urinary pH. Three healthy male Wistar rats weighing 400, 380 and 390 g respectively were starved for 24 hours and given 2 ml of 25% ammonium chloride, orally, twice a day on the day before and during the trial. Ammonium chloride has been used as urinary acidifier in drug excretion studies (Portnoff et al., 1961). A urine sample was collected from each rat before dosing and its pH was measured. Ethosuximide (40 mg) was administered and urine samples collected at 3, 6, 19, 27, 43, 66, 78, and 92 hours following oral dosing and free drug analyzed as described previously.

c) Urinary excretion study of ethosuximide under controlled alkaline urinary pH. Procedure was the same as under (b) above using rats weighing 390, 400 and 380 g except that instead of ammonium chloride, 2 ml of 25% sodium bicarbonate slurry was administered to rats, orally, twice a day on the day before and during trial to render the urine alkaline.

d) Urinary excretion study of ethosuximide under influence of probenecid treatment. Three healthy rats weighing 400, 420 and 444 g were starved as under (a) and treated with 100 mg of probenecid, orally, (2 ml of 5% suspension), twice a day on the day before and during trial. The rats were dosed orally with 40 mg of ethosuximide as under (a), (b) and (c) and urine samples collected at 3, 6, 22, 33.5, 47.5 and 75 hours following oral dosing. Blank urine samples were collected before dosing. Unchanged ethosuximide was determined in urine as described previously.

The pH of the urine of uncontrolled (untreated) rats or rats treated with probenecid (250 mg/kg) was 6.4 - 7.0; and the pH of the urine of rats treated with ammonium chloride ranged between 5.0 - 5.5; while the pH of the urine of rats treated with sodium bicarbonate was 8.0 - 8.3. The urinary excretion data from the rats in each treatment group are presented in Tables A-21 to A-33. Figure 25 shows the typical cumulative urinary excretion of unchanged ethosuximide as a function of time in a rat from each treatment group. Table XXII shows the mean cumulative urinary excretion of ethosuximide in rats under uncontrolled urinary pH and in rats under probenecid treatment. The mean cumulative urinary excretion of ethosuximide in rats under acid urinary pH and

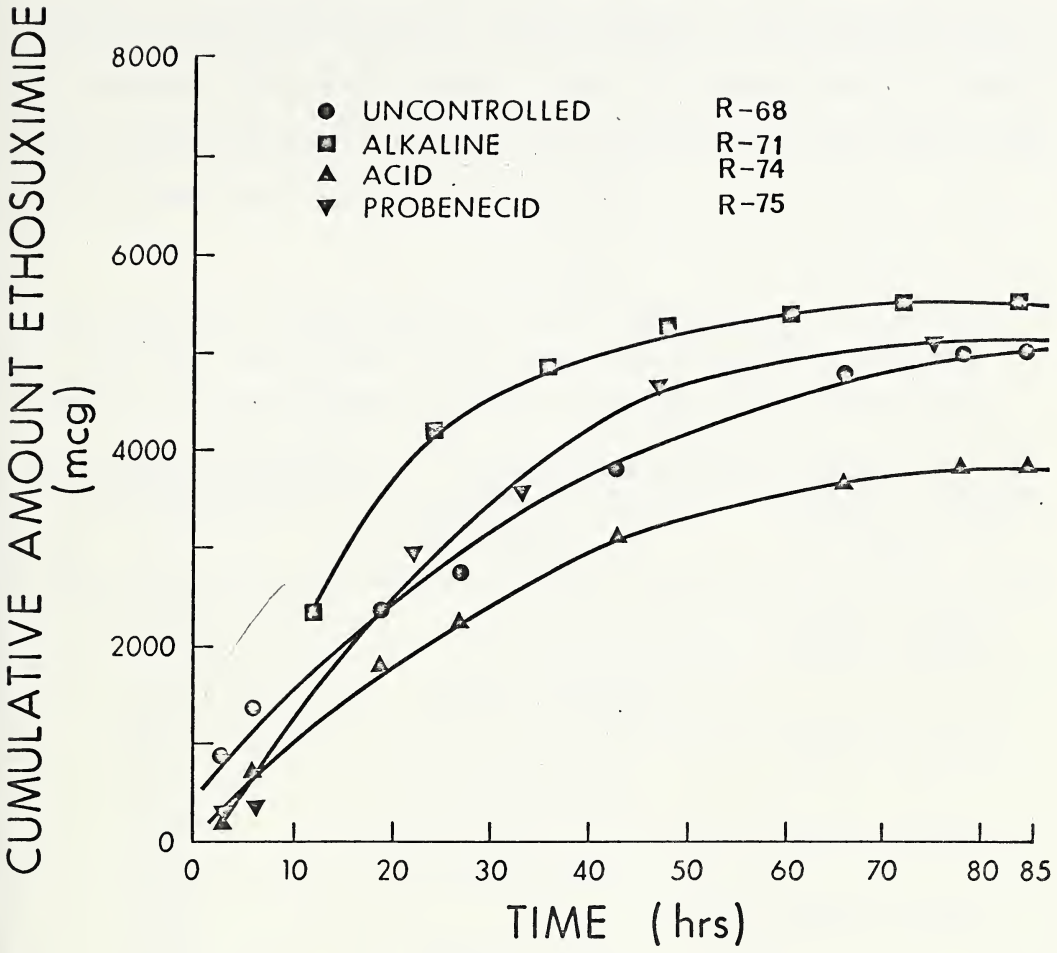

Figure 25. The cumulative urinary excretion of unchanged ethosuximide under various treatments.

under alkaline urinary pH is shown in Table XXIII. Figure 26 illustrates the cumulative amount of unchanged ethosuximide excreted in the urine following the oral dose of each treatment. The cumulative amount of unchanged ethosuximide excreted in the urine was obtained by successively adding the amount of ethosuximide excreted at various times to the total amount excreted at the previous time.

TABLE XXII

Mean cumulative urinary excretion data for ethosuximide in male Wistar rats under uncontrolled urinary pH and under probenecid treatment
Dose = 40 mg ethosuximide solution in dextrose saline injection USP

Urine Sample Collection Time Following Dose (hr)	Ethosuximide Excreted Unchanged Uncontrolled Treatment pH (6.4 - 7.0) (mcg)	Probenecid Treatment pH (6.4 - 7.0) (mcg)
3.0	-	507.7 ± 593.0
6.0	-	941.0 ± 878.0
12.5	2684.0 ± 359.0[a]	-
22.0	-	3270.0 ± 725.0
24.5	4181.0 ± 302.0	-
33.5	-	3885.0 ± 630.0
36.0	5103.0 ± 706.0	-
47.5	-	4593.0 ± 725.0
48.5	5635.0 ± 807.0	-
60.5	5869.0 ± 882.0	-
72.5	6020.0 ± 908.0	-
75.0	-	4875.0 ± 731.0
84.5	6085.0 ± 929.0	-
98.0	6153.0 ± 976.0	-
Mean Percent Dose Excreted	15.38	12.18

[a]Standard deviation (N = 3)

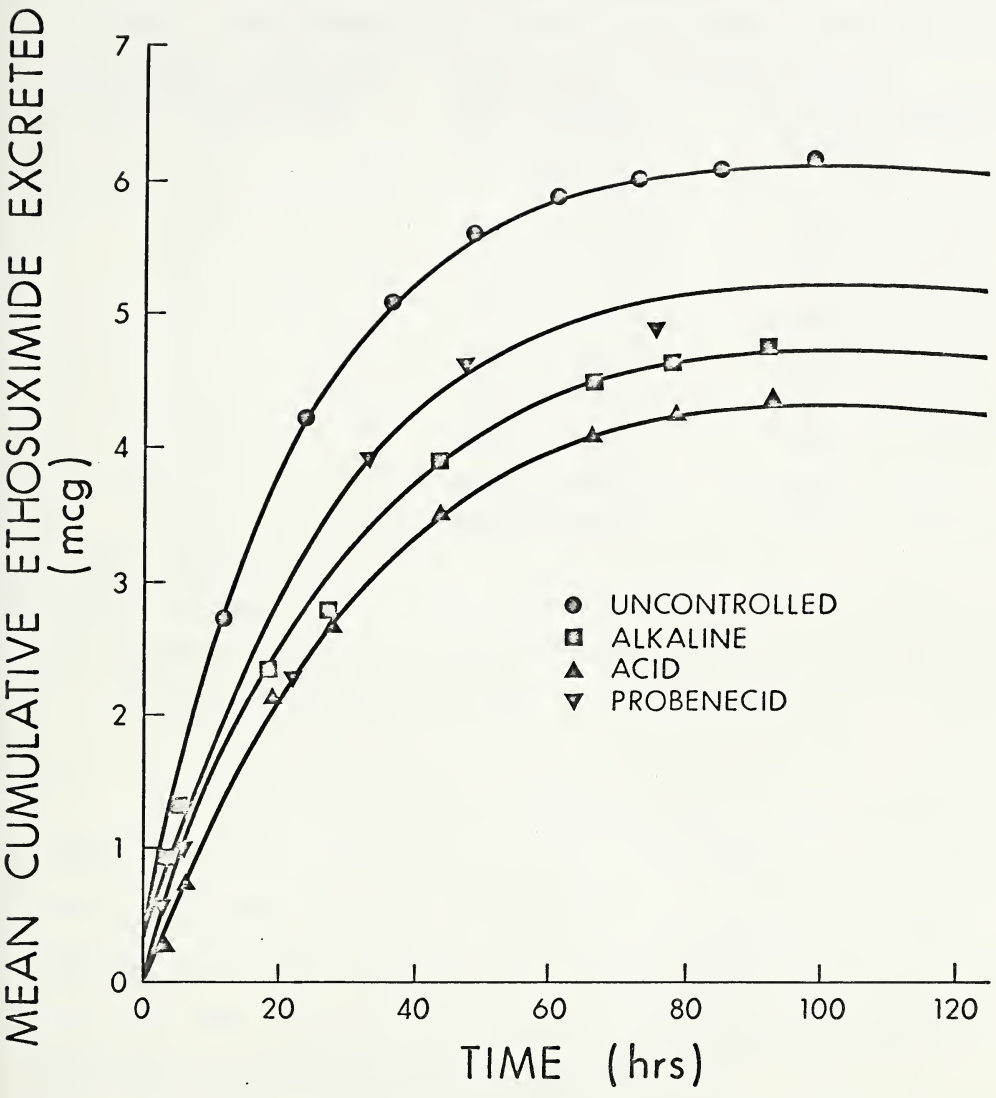

Figure 26. Mean cumulative unchanged ethosuximide excreted in the urine under various treatments.

TABLE XXIII

Mean cumulative urinary excretion data for ethosuximide in male Wistar rats under acid and alkaline treatments

Dose = 40 mg ethosuximide solution in dextrose saline USP

Urine Sample Collection Time Following Dose (hr)	Ethosuximide Excreted Unchanged Acid Treatment pH (5.0 - 5.5) (mcg)	Alkaline Treatment pH (8.0 - 8.3) (mcg)
3.0	212.6 ± 29.53[a]	895.6 ± 64.5
6.0	706.0 ± 33.04	1256.5 ± 160.3
19.0	2136.3 ± 355.0	2285.0 ± 251.8
27.0	2633.3 ± 377.0	2690.0 ± 207.5
43.0	3519.0 ± 452.0	3880.0 ± 932.0
66.0	4047.0 ± 477.0	4445.0 ± 1189.0
78.0	4233.0 ± 489.0	4607.0 ± 1211.0
92.0	4347.0 ± 485.0	4741.0 ± 1232.0
Mean Percent Dose excreted	10.86	11.85

[a]Standard deviation (N = 3)

The urinary excretion rate was obtained by dividing the amount of ethosuximide excreted unchanged during a given interval of time by the value of the time interval. The time for the excretion rate thus obtained was taken as the mid point of the interval (mean time). The excretion rate values and the respective mean times are listed in Table A-21 to Table A-33 for each treatment.

A typical semilogarithmic plot of excretion rate versus time for ethosuximide is shown in Figure 27 and Figure 28. Similar plots were constructed from data for each animal. The slope of the linear portion of the semilogarithmic plot was used to calculate the elimination rate

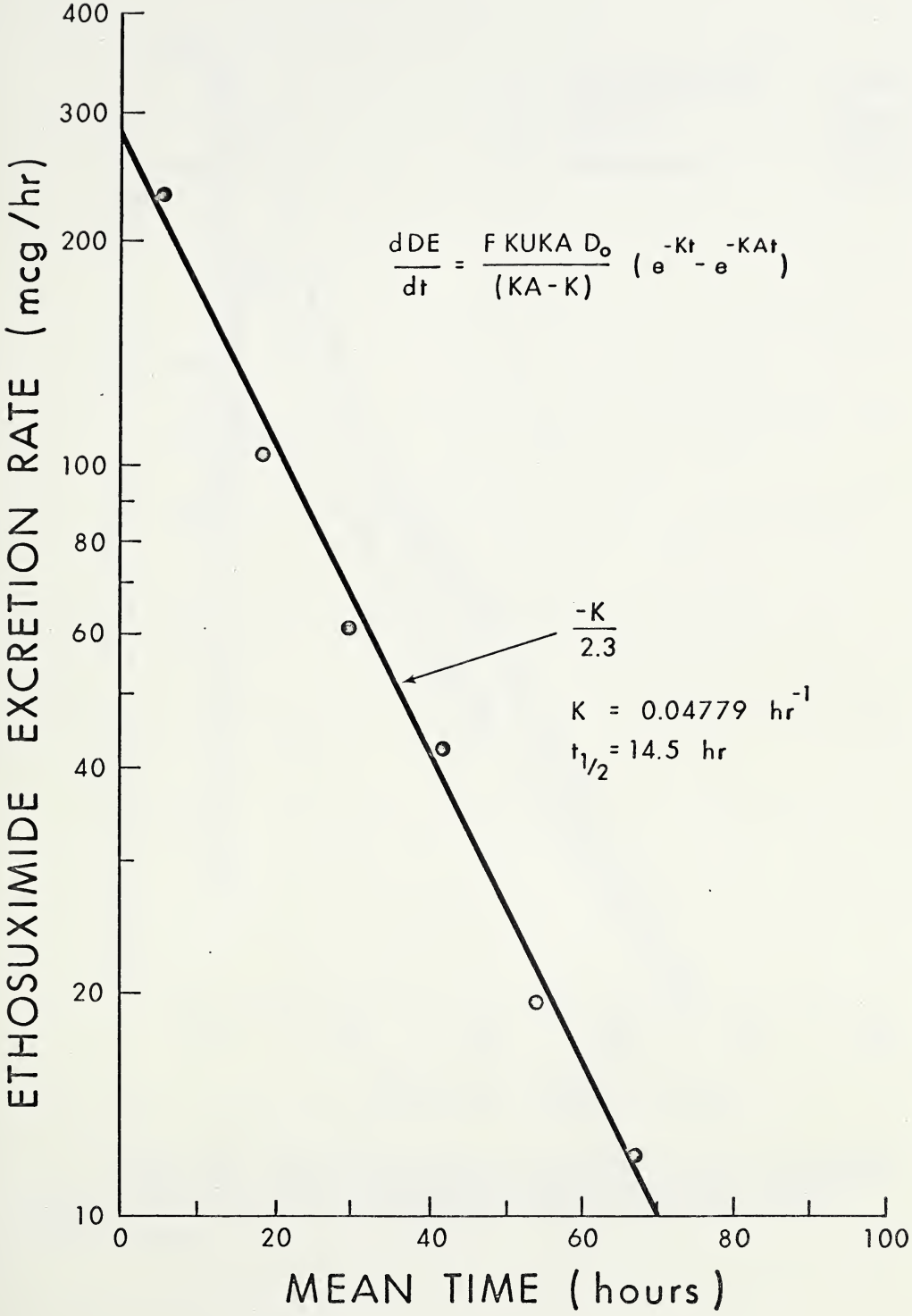

Figure 27. Semilogarithmic plot of urinary excretion rate as a function of time in Rat 68 following 40 mg oral dose of ethosuximide.

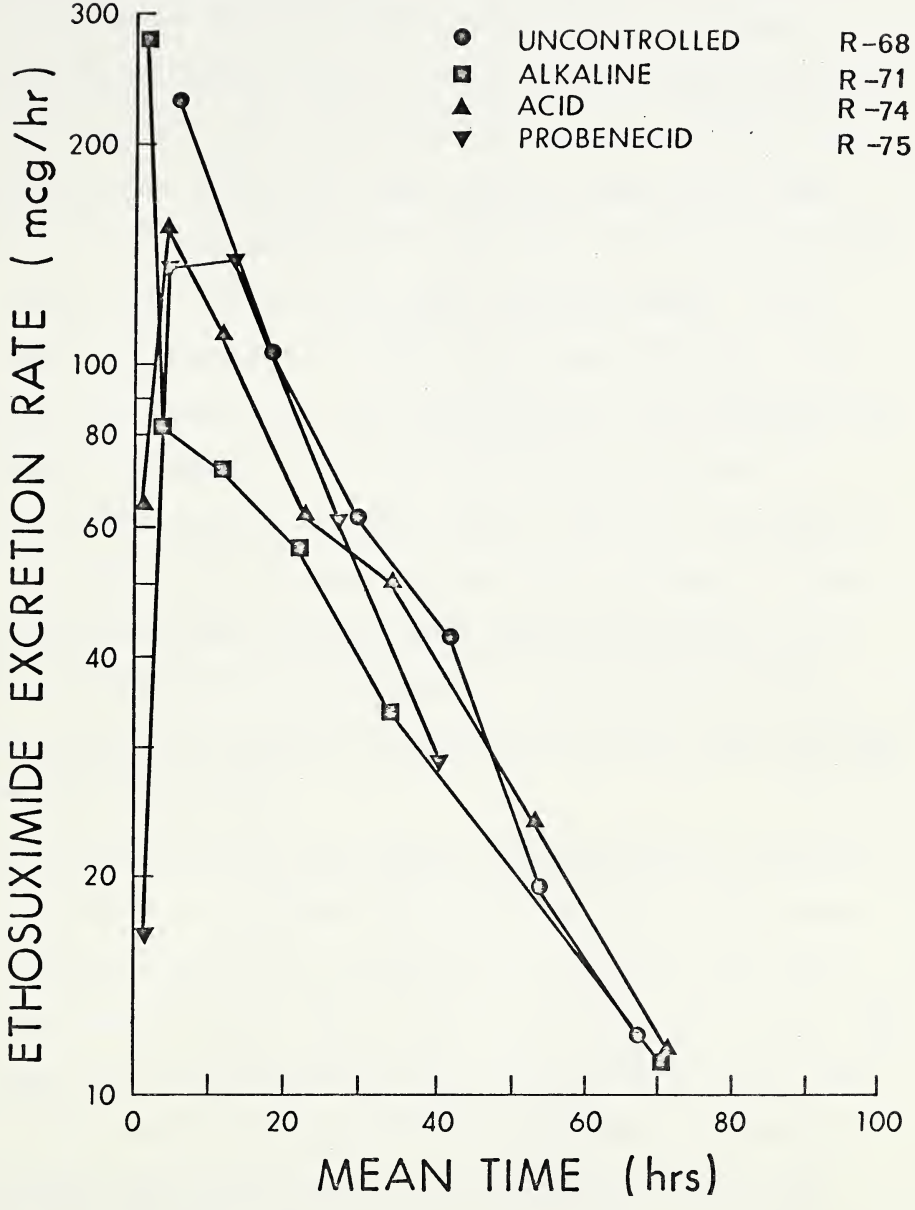

Figure 28. The urinary excretion rate-time profile of unchanged ethosuximide under various treatments in male Wistar rats, following the oral administration of 40 mg ethosuximide in dextrose saline injection USP.

constant, K, as illustrated in Figure 27. The best fit to the linear portion of excretion rate-time points was obtained by log-linear regression analysis using a Digital PDP-8/L computer using the program listed in Appendix B. The elimination rate constant calculated from the mean excretion rate data for each treatment group was not much different from that obtained from the excretion rate data of each individual animal under that treatment group averaged together. Thus, inter-animal variation was quite small under these conditions. The elimination rate constant was also obtained from a semilogarithmic plot of percent of unchanged ethosuximide remaining to be excreted as a function of time, according to Nelson (1961b). This is illustrated in Figure 29. The percent of unchanged ethosuximide excreted was based on the cumulative amount of ethosuximide excreted unchanged at time infinity. The elimination rate constants, K, obtained by the two methods mentioned above were in close agreement with the value obtained from the blood level data.

The urinary excretion rate constant, Ku, was computed from the Y intercept value of the semilogarithmic plot of excretion rate versus time (Figure 27) using Ka, the absorption rate constant, and F, the fraction of dose absorbed, values obtained from blood level study. The Y intercept value was multiplied by (Ka-K) and divided by FDKa. The K used in this calculation was obtained from the slope of excretion rate-time plot for that particular rat, FD is the fraction of dose absorbed. The excretion rate constant, Ku, was also calculated from the relationship: $Ku = (DE)_{\infty}.K/FD$. The values of Ku obtained by these two methods were similar. The $(DE)_{\infty}$ value was obtained from the mean cumulative plot (Figure 26). The clearance of unchanged

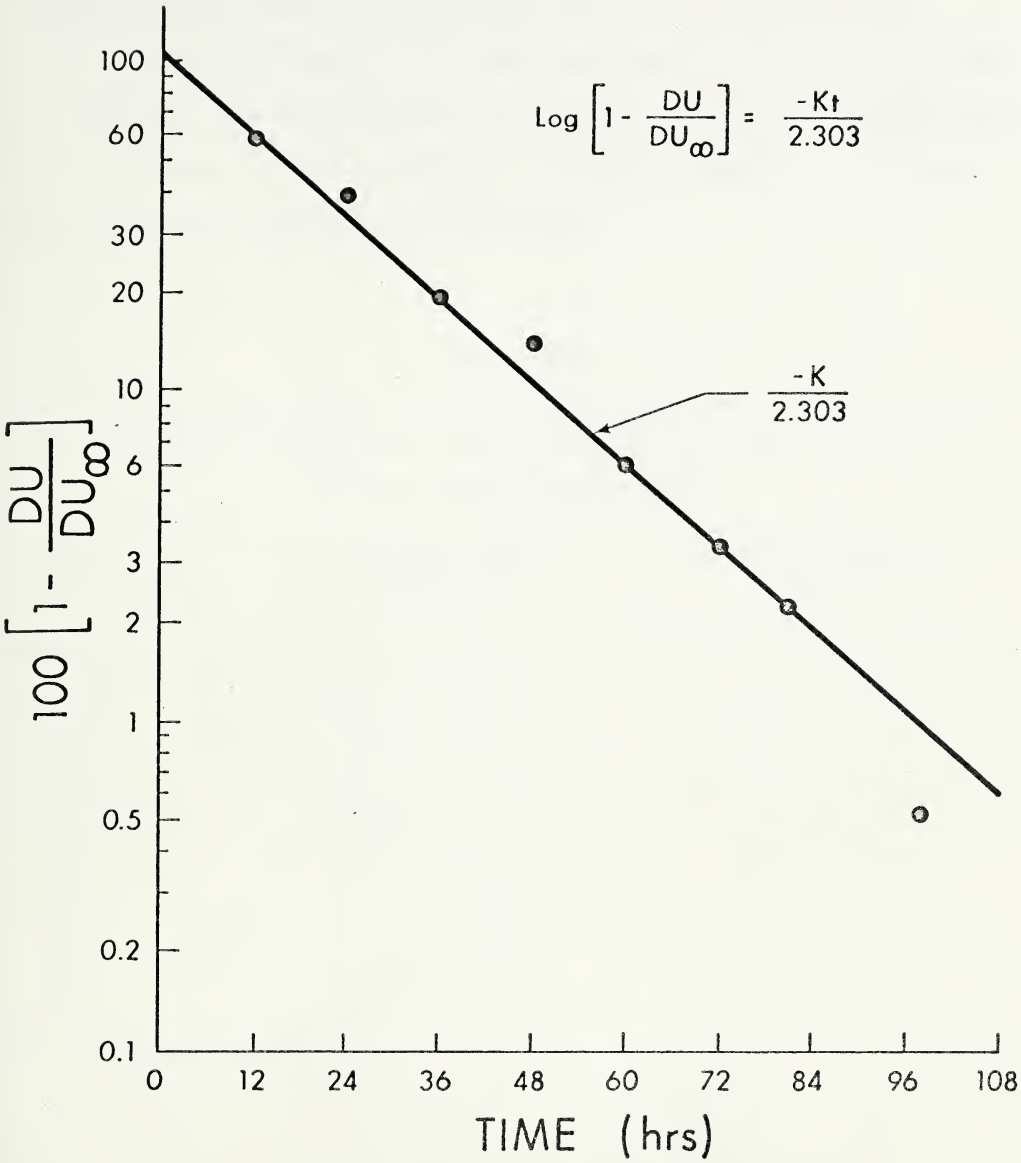

Figure 29. Percent of unchanged ethosuximide remaining to be excreted as a function of time following 40 mg oral dose to Rat 64.

ethosuximide was determined from the slope of the excretion rate versus plasma concentration data obtained from six rats, as illustrated in Figure 30. Table XXIV shows the data used for clearance calculation. Various pharmacokinetic parameters for urinary excretion of unchanged ethosuximide are summarized in Table XXV.

TABLE XXIV

Mean plasma concentration and mean urinary excretion rate of unchanged ethosuximide in male Wistar rats following oral administration of 40 mg ethosuximide solution.

Time (hr)	Excretion Rate (mcg/hr) N = 3	Plasma Concentration (mcg/ml) N = 3
6.25	230.0 ± 27.0[a]	90.33 ± 18.8
18.5	119.82 ± 23.8	41.07 ± 6.2
30.25	104.53 ± 42.46	19.35 ± 2.1
42.25	47.86 ± 10.36	8.98 ± 1.0
54.5	33.25 ± 25.0	4.1 ± 0.5
66.5	19.47 ± 13.29	1.9 ± 0.2
78.7	8.43 ± 4.75	0.87 ± 0.1
89.5	6.61 ± 4.03	0.44 ± 0.05

[a]Standard deviation.

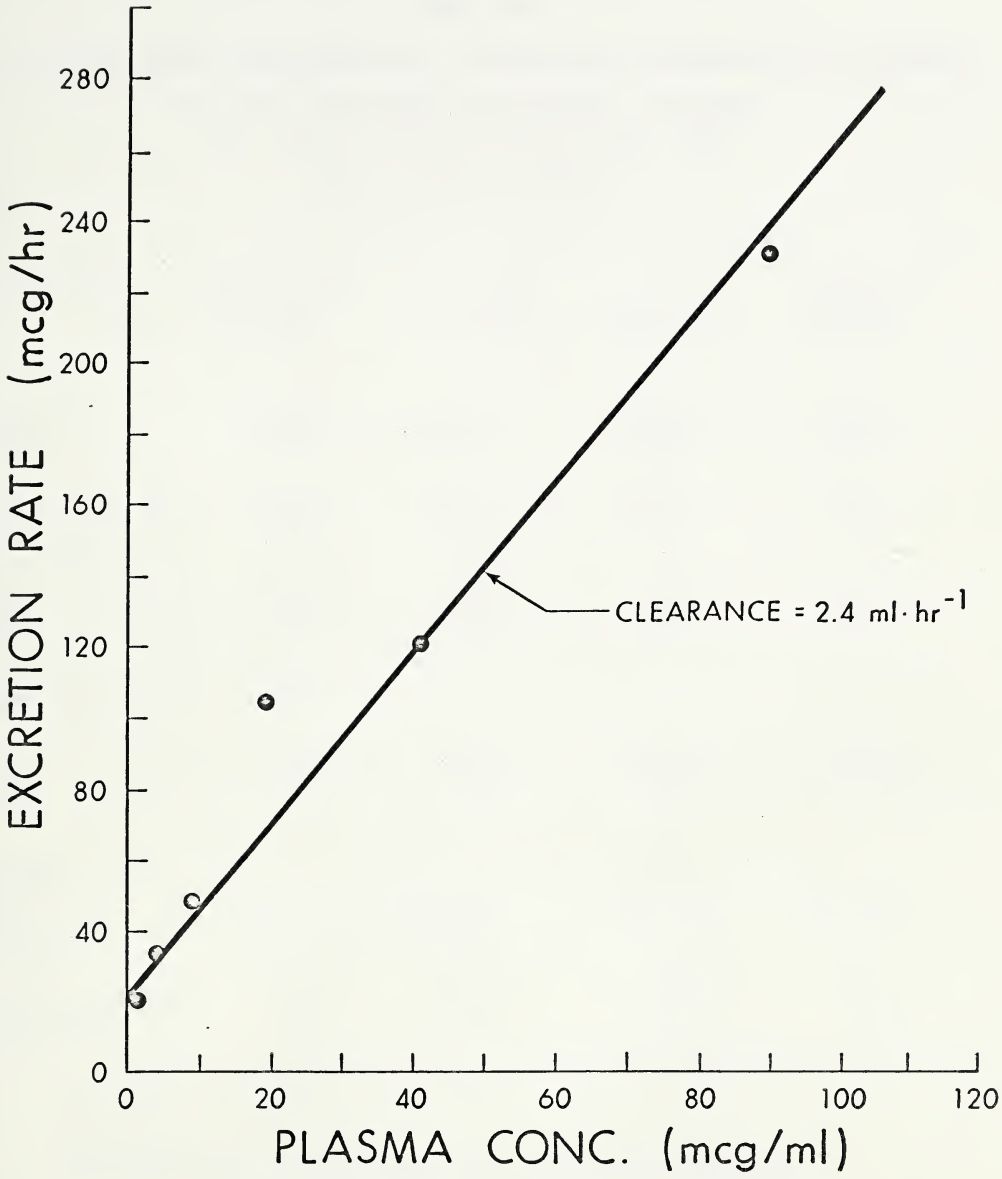

Figure 30. Urinary excretion rate of unchanged ethosuximide as a function of plasma concentration of unchanged ethosuximide. Each point is mean of the data from six rats.

TABLE XXV

Urinary excretion pharmacokinetic parameters of ethosuximide, according to a two compartment open model, following oral administration to male Wistar rats
Dose = 40 mg solution

Parameters	Uncontrolled Treatment N = 3	Alkaline Treatment N = 3	Acid Treatment N = 3	Probenecid Treatment N = 3
Ku (hr^{-1})	0.008 ± 0.001	0.004 ± 0.001	0.004 ± 0.001	0.007 ± 0.002
Maximum Excretion Rate	220.0 ± 13.8	298.0 ± 12.4	164.5 ± 5.6	232.0 ± 80.6
Peak Excretion Time	6.25 ± 0.14	1.5 ± 0.0	4.5 ± 0.0	10.83 ± 5.4
Ka[b] (hr^{-1})	1.97 ± 0.93	-	-	-
$T_{1/2}$ (hr)	13.8 ± 2.3	19.6 ± 1.63	19.74 ± 0.86	12.98 ± 1.69
FDKaKu / Ka - K (mcg/hr)	310.12 ± 29.53	167.55 ± 28.23	161.33 ± 6.04	284.96 ± 68.24
% Dose Excreted Unchanged	15.4 ± 2.4	11.9 ± 3.1	10.9 ± 1.2	12.2 ± 1.85

[a]Standard deviation.

[b]From blood level study.

-No blood level study done under particular treatments.

7. TISSUE DISTRIBUTION STUDIES OF ETHOSUXIMIDE

Three male Wistar rats 272, 384 and 392 g respectively were anesthetized and cannulated as described previously and 40 mg ethosuximide (1 ml) was administered to each rat through the femoral vein. Rats were sacrificed by chloroforming at 3, 7 and 21 hours following the dose. Samples of tissue from skeletal muscle, liver, brain, fat, GIT, heart, lung, whole kidney and kidney cortex were quickly removed, weighed, and homogenized with physiological saline to make 10 to 15 ml of homogenate. One ml of the homogenate was analyzed for free ethosuximide using the procedure described previously and the amount of ethosuximide per gramme of wet weight of tissue calculated.

The concentration of ethosuximide in one gram of the different tissues analyzed was found to vary to some extent. Table XXVI shows the concentration, in micrograms of ethosuximide, found in several different tissues. Only one rat was used for studying tissue distribution within a given time interval following the dose. This was done in an attempt to confirm the reports claiming even distribution of ethosuximide throughout the body.

TABLE XXVI

Tissue distribution of ethosuximide in rats following I.V. administration under general anesthesia.

Tissue	Ethosuximide level (mcg/g) Time after dosing. A 3 hr	B 7 hr	C 21 hr
Skeletal muscle	106.6	143.5	61.4
Liver	146.4	85.2	103.3
Brain	187.5	90.4	108.9
Fat	147.5	56.2	82.6
GIT	72.7	107.5	92.4
Heart	132.4	230.5	104.9
Lung	195.9	125.3	193.6
Cortex (Kidney)	167.8	137.2	142.6
Kidney	134.3	116.7	117.4

A = Data from single rat weighing 384 g, B = Data from single rat weighing 272 g, C = Data from single rat weighing 392 g.

8. PHARMACOKINETIC MODELS AND COMPUTATIONS

Pharmacokinetic models described previously were used to describe the real physiological phenomena associated with the absorption, distribution, metabolism and excretion of the drug. Simple differential equations were written in an attempt to account for all the drug changes (biotransformation and/or redistribution) from the time of dosage administration to the time of the total elimination of the drug. Based on data from the blood level study, following intravenous injection of ethosuximide to rats, a two compartment pharmacokinetic model was used to evaluate the urinary excretion of the drug (Figure 31). A compartment may be defined as a kinetically distinguishable pool in terms of the drug concentration time profile. A two compartment model has been used by a number of investigators (Teorell, 1937b; Dominquez, 1950; Solomon, 1960; Riggs, 1963; Nelson and Kruger-Thiemer, 1964; Rescigno and Segre, 1966 and Riegelman et al., 1968). The following assumptions were made for the use of a two compartment model:

1. The rate of urinary excretion of the drug is proportional to its plasma concentration.
2. Drug transfer between the central and the peripheral compartment is reversible, but transfer from the central compartment to the urinary compartment is irreversible.
3. The rates of absorption, distribution and metabolism follow first order kinetic processes.
4. Drug elimination occurs from the central compartment.
5. Excretion of unchanged drug and its metabolite occurred entirely from kidney.

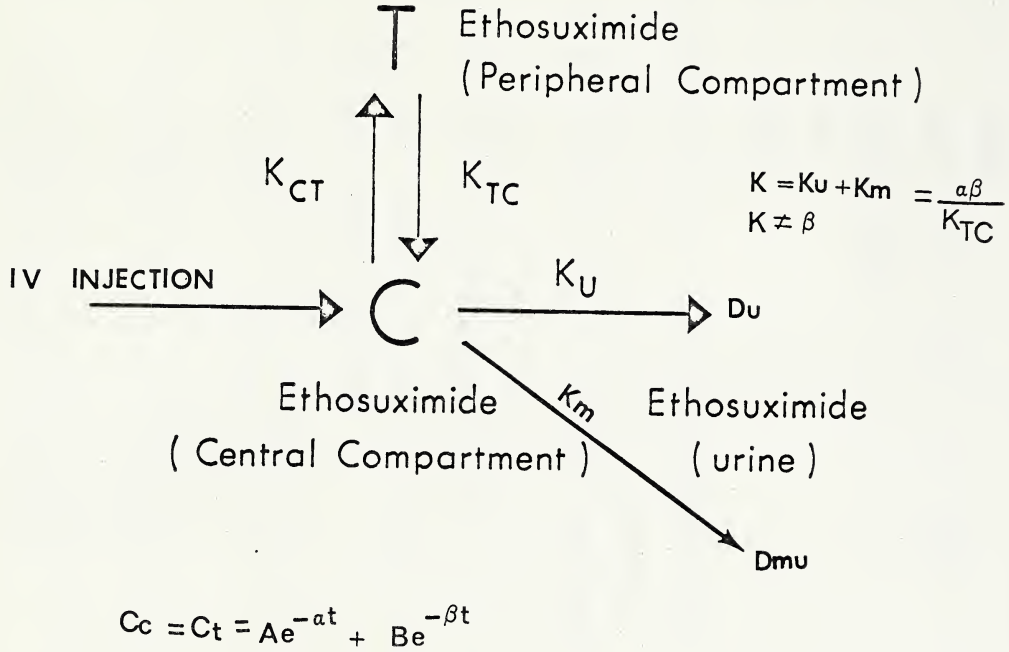

Figure 31. A scheme of the two compartment pharmacokinetic model for ethosuximide blood level or urinary excretion following intravenous administration to Wistar rats.

The following differential equations defined the pharmacokinetic model proposed:

$$dDg/dt = -KaDg \quad (37)$$

$$dDc/dt = KaDg + KtcDt - KctDc - KuDc - KmDc \quad (38)$$

$$dDt/dt = KctDc - KtcDt \quad (39)$$

$$dDu/dt = KuDc \quad (40)$$

$$dDm/dt = KmDc \quad (41)$$

Where Dg = the amount of drug in the gastrointestinal tract:

Dc = the amount of drug in central compartment.

Dt = the amount of drug in peripheral compartment.

Du = the amount of intact drug excreted in the urine.

Dm = the amount of metabolites excreted in the urine.

Ka = the rate constant for the absorption of drug from the GIT into central compartment.

Ktc = the first order rate constant for the transfer of drug from peripheral compartment to central compartment.

Kct = the first order rate constant for the transfer of drug from central compartment to peripheral compartment.

Ku = the first order rate constant for the excretion of drug from central compartment into urine.

Km = the first order rate constant for metabolism of drug in the central compartment.

t = time after administration of the dose.

Figure 31 shows the scheme and the equations that describe a two compartment pharmacokinetic model for the analysis of plasma level data following the intravenous administration of ethosuximide to rats.

In Figure 31, if the rate constants, Kct and Ktc, become equal the peripheral compartment T automatically drops out and the scheme then describes a one compartment pharmacokinetic model for an intravenous dose. Equation 42 describes the plasma level according to a one compartment open model following an intravenous dose.

$$C_c = \frac{D_0}{V} e^{-Kt} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (42)$$

where C_c is the plasma concentration of ethosuximide at any time t, D_0 is the initial dose given and K is the elimination rate constant. Since the semilogarithmic plot of plasma level data of ethosuximide, methsuximide and phensuximide (Figures 16, 17 and 18) showed a biexponential decay, the two compartment pharmacokinetic model scheme (Figure 31) was used to analyze the plasma level data following the intravenous doses. The quality of the computer fit to the experimental data was tested by using as a criterion, the sum of the squares of the deviations between the experimental data and the data calculated by the computer according to the equation describing the plasma level in a two compartment open model. This should be less than 0.01% of the observed value. An excellent fit was found using a two compartment pharmacokinetic model. Addition of a third compartment to the scheme in Figure 31 and use of equation 43 for the prediction of plasma level data did not improve the quality of the fit.

$$C_c = Ae^{-\alpha t} + Be^{-\beta t} + Ce^{-\gamma t} \quad . \quad . \quad . \quad . \quad (43)$$

where C_c is the plasma concentration at any time t following an intravenous dose, α, β and γ are the various disposition rate constants for triexponential decay of plasma level concentrations of drug. Figure 32 shows the scheme for a 'two' compartment (one compartment)

$$Cc = \frac{FDK_A}{V(K_A - K)}\left(e^{-Kt} - e^{-K_A t}\right)$$

$$\frac{dU}{dt} = \frac{K_u FDK_A}{K_A - K}\left(e^{-Kt} - e^{-K_A t}\right)$$

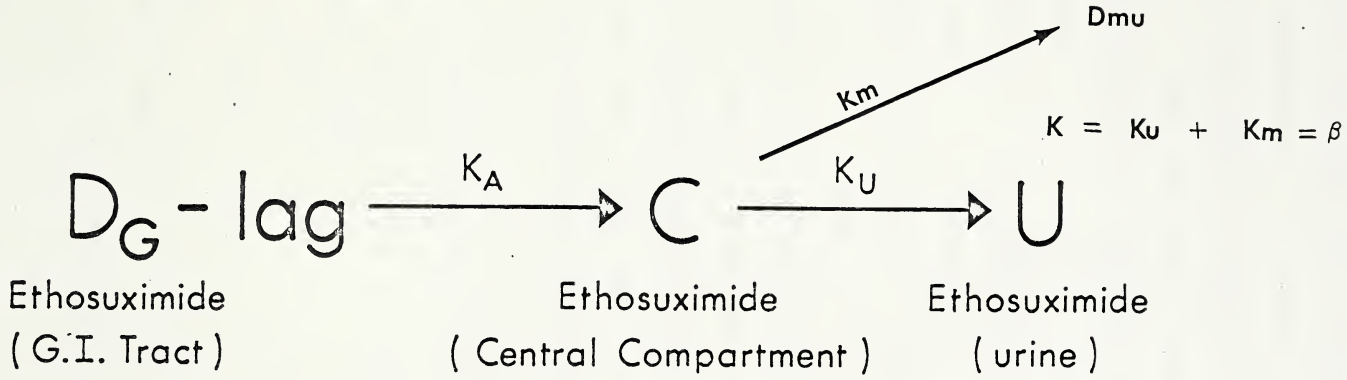

Figure 32. A scheme showing a 'two' compartment pharmacokinetic model for ethosuximide blood level or urinary excretion following oral administration to Wistar rats.

pharmacokinetic model used to analyze the plasma level data following an oral dose. Figure 33 illustrates the two compartment pharmacokinetic model scheme for the analysis of plasma level data following multiple oral dosing. The equations describing the plasma level according to the two proposed pharmacokinetic models were used to predict the plasma level data and the difference between the sum of the squares of the deviations between the experimental data and the data predicted according to particular model was compared. A 'two' compartment pharmacokinetic model was found to adequately describe the pharmacokinetics of ethosuximide in rats following single oral administration. Though this model is really one compartment, it is usually referred to as a 'two' compartment model in most literature because of the absorption and elimination processes involved in the pharmacokinetics following the oral dose. Therefore, in this study this one compartment pharmacokinetic model has been described as a 'two' compartment model for the oral blood level data.

The integrated equations describing the plasma level-time relationship following an intravenous dose in two compartment open model (Equation 14) and that following oral dose in one compartment open model (Equation 7) were properly defined in subroutine 'DFUNC' of the program 'NONLIN' (Metzler, 1968).

$$Cc = Ae^{-\alpha t} + Be^{-\beta t} \quad \ldots\ldots\ldots\ldots \quad (14)$$

$$Cc = \frac{FKaD}{V\,(Ka-K)}\,(e^{-Kt} - e^{-Kat}) \quad \ldots\ldots \quad (7)$$

where Cc = the plasma concentration of drug at time t.

A and B = The y intercepts on semilogarithmic plot of the plasma concentration versus time (Figures 16 and 19).

α = Fast disposition rate constant.

β = Slow disposition rate constant.

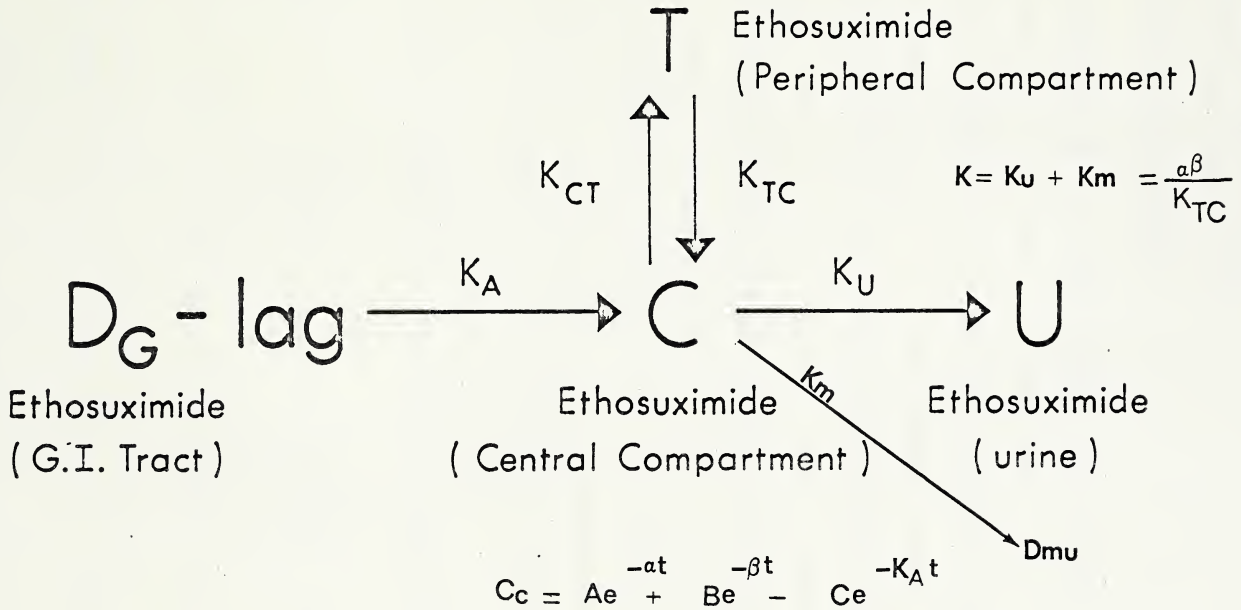

Figure 33. Scheme showing a two compartment pharmacokinetic model for ethosuximide blood levels or urinary excretion following the oral administration to Wistar rats.

t = time.

F = fraction of dose absorbed.

Ka = absorption rate constant.

K = elimination rate constant.

V = volume of distribution.

The initial estimates of α, β, AB, and $\frac{FD}{V}$ = Ao, Ka and K were determined graphically as illustrated in Figures 16 and 19.

The initial estimates along with upper and lower limits of the estimates and observed values of Y, the plasma concentration and X, the time, were read into an IBM 360 computer through subroutine 'DFUNC' (Figures 34 and 35) which called the main program and performed the iterative least squares fitting of the plasma level-time curves. Typical digital computer simulation and pharmacokinetic parameter estimations are shown using the plasma level data of Rat 1 from Table A-1. Figure 35 shows the iteration part which follows the input of initial estimates of pharmacokinetic parameters and the plasma level-time data through subroutine 'DFUNC'. Figure 36 shows the output of subroutine SUMARY which summarizes the best estimates of the pharmacokinetic parameters together with other derived pharmacokinetic constants and parameters after the iterations converge. The criterion for convergence was defined along with the data input so that when the sum of squared deviations between the observed and calculated data points in subsequent iterations was less than 0.01% of the observed value the program converged. Figure 37 shows the output of subroutine 'EIGEN' which computes the eigenvalues and eigenvectors of the correlation matrix of the estimates. This

```
0001                SUBROUTINE DFUNC(T,I,J,K,P,VAL)
                C
                C       TWO COMPARTMENTAL OPEN MODEL FOR RAPID IV INJECTION
                C
0002                COMMON NP,NC,NIV,NDE,NIT,NTP, PM, IP,IT,SS,WS
0003                COMMON PL(32), CON(8),IW(10),NPT(11),Y(10),IDIG(10)
0004                COMMON NAME, LPLOT,LDEO,TEST,CEL
0005                COMMON YOBS(400),X(800),DV(400),W(400),YCALC(400)
0006                DIMENSION P(1),VAL(1),NAME(10)
                C
                C       THE FOLLOWING DEFINES THE FUNCTION FOR BLOOD DATA IV
                C
0007                P1=P(1)
0008                P2=P(2)
0009                P3=P(3)
0010                P4=P(4)
0011                DO=CON(1)
0012                XT=X(I)
0013                E1=EXP(-P2*XT)
0014                E2=EXP(-P4*XT)
0015          10    T=P1*E1+P3*E2
0016                GO TO 200
                C
                C       THE FOLLOWING STATEMENTS PROVIDE CALCULATED PARAMETERS
                C
0017          200   IF(IP-9) 40,30,40
0018          30    TK1=((P2*P4)*(P1+P3))/(P1*P4+P3*P2)
0019                TK2=((P1*P3)*(P4-P2)**2)/((P1+P3)*(P1*P4+P3*P2))
0020                TK3=(P1*P4+P3*P2)/(P1+P3)
0021                TK4=DO/(P1+P3)
0022                TK5=(TK4*TK2)/TK3
0023                TK6=((TK2*DO)/(P2-P4))*(E2-E1)
0024          21    TK8=T*TK4
0025                TK9=(-ALOG(.5)/P4)/60.
0026                AUC=(P1/P2)+(P3/P4)
0027                WRITE(6,1) TK1,TK2,TK3,TK4,TK5
0028          1     FORMAT('  K =',F7.6,'  KCT= ',F7.6,' KTC= ',F7.6,'  VC= ',F12.1,
                     X'VT = ',F12.1)
0029                WRITE(6,2)TK6,TK8,TK9
0030            2   FORMAT('DT =',F15.1,' DC =',F15.1,' THF=',F10.3)
0031                WRITE(6,3)AUC
0032            3   FORMAT('AUC= ',F15.1)
                C       WHERE P1 IS A,P2 IS  ALPHA,P3IS B,P4 IS BETA,DO IS DOSAE
                C       TK1 IS K,TK2 IS KCT,TK3 IS KTC,TK4 IS VC,TK5 IS VT,TK6 IS DT,
                C       TK8 IS DC,TK9 IS HALF LIFE
0033          40    RETURN
0034                END
```

Figure 34. 'NONLIN' subroutine 'DFUNC' for the two compartment pharmacokinetic model following I.V. dose.

```
NONLIN.....   SZM000

 1 FUNCTION(S). NO. OF OBSERVATIONS OF EACH FUNCTION =  14.  0.  0.  0.  0.  0.  0.  0.  0.  0. TOTAL NO. OF OBSERVATIONS =  14
MAX. NO. OF ITERATIONS IS 20. LDATA=0. LIMIT=0. LPAR=0. LSPEC=0. LPLOT=0. LDFQ=0. IDIG =  -4.  0.  0.  0.  0.  0.  0.  0.  0.  0.
TEST= 0.0001. DEL= 0.00100. PM= 0.00100. THERE ARE  1 CONSTANTS AND  2 DIFFERENTIAL EQUATIONS.  IW = 2,0,0,0,0,0,0,0,0,
THERE ARE  4 PARAMETERS WITH INITIAL ESTIMATES AND LIMITS AS FOLLOWS:

    NO.        INITIAL ESTIMATES      LOWER LIMITS      UPPER LIMITS

      1            52.000000          10.000000         100.000000
      2             0.044970           0.004000           0.100000
      3           120.000000          50.000000         200.000000
      4             0.000840           0.000100           0.005000

ITER-
ATION PART  WEIGHTED SS          P 1          P 2          P 3          P 4          P
   1    1  0.130415E 03    0.520E 02    0.450E-01    0.120E 03    0.840E-03
1
CNO ERROR

   1    2  0.101397E 03    0.525E 02    0.516E-01    0.122E 03    0.875E-03
   1    3  0.910712E 02    0.531E 02    0.583E-01    0.123E 03    0.909E-03
   1    4  0.910670E 02    0.531E 02    0.587E-01    0.124E 03    0.911E-03
   2    1  0.910670E 02    0.531E 02    0.587E-01    0.124E 03    0.911E-03
1
CNO ERROR

   2    2  0.890410E 02    0.536E 02    0.603E-01    0.123E 03    0.908E-03
   2    3  0.882650E 02    0.541E 02    0.618E-01    0.123E 03    0.905E-03
   2    4  0.882533E 02    0.542E 02    0.620E-01    0.123E 03    0.905E-03
   2    1  0.882533E 02    0.542E 02    0.620E-01    0.123E 03    0.905E-03
1
CNO ERROR

   3    2  0.882383E 02    0.542E 02    0.623E-01    0.123E 03    0.906E-03
   3    3  0.882338E 02    0.542E 02    0.625E-01    0.123E 03    0.907E-03
   3    4  0.882331E 02    0.542E 02    0.625E-01    0.123E 03    0.907E-03
   4    1  0.882331E 02    0.542E 02    0.625E-01    0.123E 03    0.907E-03
1
CNO ERROR

   4    2  0.882348E 02    0.542E 02    0.625E-01    0.123E 03    0.907E-03
   4    3  0.882336E 02    0.542E 02    0.625E-01    0.123E 03    0.907E-03
   4    4  0.882323E 02    0.542E 02    0.625E-01    0.123E 03    0.907E-03
```

Figure 35. Iteration part of program 'NONLIN' during least-square fit of plasma level data from Rat - 1 following I.V. dose of ethosuximide (40 mg).

SUMMARY OF NON-LINEAR ESTIMATION

SZM000

AFTER 4 ITERATIONS THE ESTIMATES AND THEIR VARIABILITY ARE:

NO.	ESTIMATE	STD. DEV.	95% CONFIDENCE LIMITS	
1	0.541803E 02	0.302331E 01	0.474439E 02	0.609167E 02 UNIVAR
			0.427593E 02	0.656013E 02 S PLANE
2	0.624906E-01	0.106252E-01	0.388161E-01	0.861650E-01 UNIVAR
			0.223525E-01	0.102629E 00 S PLANE
3	0.123337E 03	0.230147E 01	0.118209E 03	0.128465E 03 UNIVAR
			0.114043E 03	0.132031E 03 S PLANE
4	0.906996E-03	0.616082E-04	0.769724E-03	0.104427E-02 UNIVAR
			0.674262E-03	0.113973E-02 S PLANE

K =.001297 KCT= .018406 KTC= .043695 VC= 225.3 VT = 94.9
DT = 1382.9 DC = 38515.3 THF= 12.737
AUC= 136851.4

Figure 36. Best estimates of pharmacokinetic parameters computed by program 'NONLIN' from plasma level-time data of Rat - 1 following I.V. dose of ethosuximide (40 mg).

-- VARIANCE-COVARIANCE MATRIX OF ESTIMATES --

0.10359E 01			
0.71362E-03	0.12795E-04		
-0.38760E 00	0.17644E-02	0.60032E 00	
-0.84682E-05	0.33619E-07	0.12365E-04	0.43018E-09

-- CORRELATION MATRIX OF ESTIMATES --

0.10000E 01			
0.19601E 00	0.10000E 01		
-0.49150E 00	0.63664E 00	0.10000E 01	
-0.40114E 00	0.45314E 00	0.76946E 00	0.10000E 01

--EIGENVALUE ANALYSIS OF ESTIMATES--

EIGENVALUES	EIGENVECTORS			
0.2398E 01	-0.3238E 00	0.4238E 00	0.6223E 00	0.5729E 00
0.1194E 01	0.7593E 00	0.6487E 00	0.7897E-03	-0.5154E-01
0.3242E 00	0.3726E 00	-0.3722E 00	-0.2887E 00	0.7995E 00
0.8386E-01	0.4240E 00	-0.5109E 00	0.7276E 00	-0.1728E 00

Figure 37. Eigenvalues and eigenvectors of the best estimates of pharmacokinetic parameters computed by program 'NONLIN' following I.V. dose of ethosuximide (40 mg) to Rat - 1.

information is only useful for seeing if any linear relations exist among the estimated parameters and if there are too many parameters in the model being fitted. Figure 38 depicts the output of subroutine 'COMPUT' which uses the best estimates of the parameters and the independent variable X (value of various time intervals) to compute the predicted values of the plasma concentration as YCALC and performs various statistics on the observed and calculated values. A value of R-Squared close to 1 and a correlation coefficient value close to 1, indicated excellent fit. Figure 39 shows the output of subroutine 'PLOTN' which makes a plot of predicted plasma concentrations YCALC as well as observed plasma concentration, YOBS versus X the time. Visual inspection of the graph was also used in judging the quality of the fit. Subroutine 'DFUNC' for digital computer simulation and parameter estimations from plasma level data according to two compartment open model (Figure 32) following oral dose are shown in Figure 40. Plasma level data of Rat 32 from Table A-9 has been used to illustrate the digital computer simulation and the parameter estimations in the case of oral blood level-time data. Figures 41 - 44 illustrate the output of the various subroutines discussed under intravenous blood level data. All the rate constants and derived pharmacokinetic parameters from I.V. and oral blood level data reported in this study were determined for each animal data as described above, by non-linear Digital Computer least-square iterations. Equations 15 - 25 were used to calculate the derived pharmacokinetic parameters for the scheme shown in Figure 32. The symbols are defined in Appendix B.

FUNCTION 1

X	OBS. Y	CALC. Y	OBS-CALC	% DEVIATION	WEIGHT
0.20000E 01	0.17310E 03	0.17093E 03	0.21714E 01	1.25	0.10000E 01
0.30000E 01	0.16850E 03	0.16792E 03	0.57950E 00	0.34	0.10000E 01
0.40000E 01	0.16650E 03	0.16509E 03	0.14121E 01	0.85	0.10000E 01
0.50000E 01	0.15550E 03	0.16242E 03	-0.69201E 01	-4.45	0.10000E 01
0.10000E 02	0.15480E 03	0.15123E 03	0.35729E 01	2.31	0.10000E 01
0.20000E 02	0.13490E 03	0.13665E 03	-0.17459E 01	-1.29	0.10000E 01
0.40000E 02	0.12520E 03	0.12339E 03	0.18082E 01	1.44	0.10000E 01
0.60000E 02	0.11820E 03	0.11808E 03	0.12050E 00	0.10	0.10000E 01
0.90000E 02	0.11570E 03	0.11386E 03	0.18352E 01	1.59	0.10000E 01
0.12000E 03	0.10780E 03	0.11065E 03	-0.28479E 01	-2.64	0.10000E 01
0.24000E 03	0.98000E 02	0.99210E 02	-0.12104E 01	-1.24	0.10000E 01
0.36000E 03	0.90000E 02	0.88979E 02	0.10206E 01	1.13	0.10000E 01
0.54000E 03	0.76000E 02	0.75576E 02	0.42360E 00	0.56	0.10000E 01
0.72000E 03	0.64000E 02	0.64192E 02	-0.19230E 00	-0.30	0.10000E 01

SUM OF SQUARED OBSERVATIONS = 0.2346487E 06
SUM OF SQUARED DEVIATIONS = 0.8823233E 02
SUM OF WEIGHTED SQUARED DEVIATIONS = 0.8823233E 02
R-SQUARED = 1.000 COR= 0.997
S = 2.9703932 WITH 10 D.F.

Figure 38. Observed and predicted values of plasma level by program 'NONLIN' according to a two compartment open model following I.V. dose of ethosuximide (40 mg) to Rat - 1.

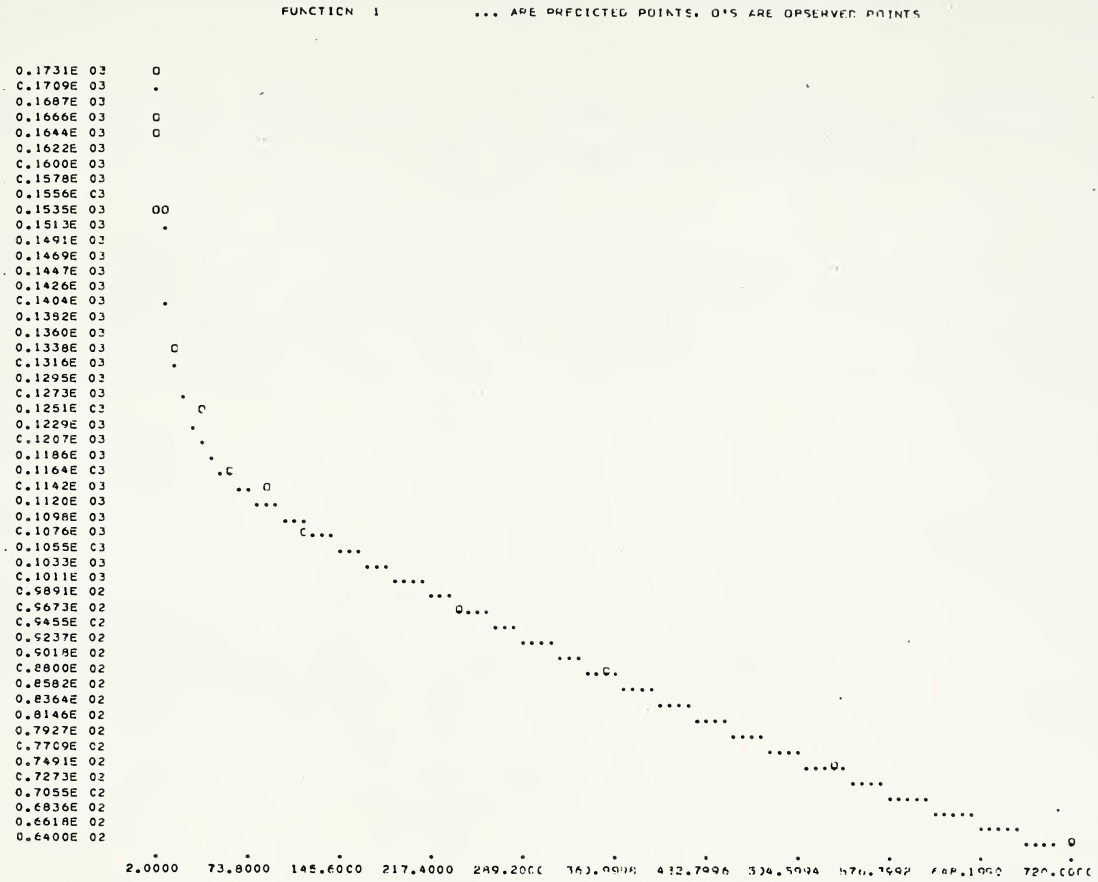

Figure 39. 'NONLIN' least-squares fit to the plasma level data following I.V. administration of ethosuximide (40 mg) solution in Rat - 1.

```
MTS  FORTRAN IV G COMPILER (C/S REL 21.6)          MAIN          02-23-74      17:29.12      PAGE 0001

            C     TWO COMPARTMENT OPEN MODEL FOR PLASMA LEVEL AFTER ORAL DOSE
            C
0001                SUBROUTINE DFUNC(T,I,J,K,P,VAL)
            C
0002                COMMON NP,NC,NIV,NDE,NIT,NTP, PM, IP,IT,SS,WS
0003                COMMON PL(32), CON(8),IW(10),NPT(11),Y(10),IDIG(10)
0004                COMMON NAME, LPLOT,LDEG,TEST,DEL
0005                COMMON YOBS(400),X(800),DV(400),W(400),YCALC(400)
0006                DIMENSION P(1),VAL(1),NAME(10)
            C
            C     THE FOLLOWING DEFINES THE FUNCTION FOR BLOOD DATA
            C
            C
0007                P1=P(1)
0008                P2=P(2)
0009                P3=P(3)
0010                XT=X(I)
0011                P23=P2-P3
0012                E1=EXP(-P3*XT)
0013                E2=EXP(-P2*XT)
0014              10  T=P1*(P2/P23)*(E1-E2)
0015                GO TO 200
            C     THE FOLLOWING STATEMENTS PROVIDE CALCULATED PARAMETERS
0016              200 IF(IP-9) 40,30,40
0017            30    TK1=-ALOG(.5)/P3
0018                  TK2=-ALOG(.5)/P2
0019                  WRITE(6,1)TK1,TK2
0020              1   FORMAT('TK1=',F10.2,'TK2=',F10.2)
0021            40    RETURN
0022                  END
```

Figure 40. 'NONLIN' subroutine 'DFUNC' for a 'two' compartment pharmacokinetic model following oral dose.

```
NONLIN.....   SZM000

 1 FUNCTION(S). NO. OF OBSERVATIONS OF EACH FUNCTION =   8.  0.  0.  0.  0.  0.  0.  0.  0.  0.  0.  0. TOTAL NO. OF OBSERVATIONS =   8
MAX. NO. OF ITERATIONS IS 20. LDATA=0. LIMIT=0. LPAR=0. LSPEC=0. LPLOT=0. LDFQ=0. IDIG = -4.  0.  0.  0.  0.  0.  0.  0.  0.  0.
TEST= 0.0001. DEL= 0.00100. PM= 0.00100. THERE ARE  0 CONSTANTS AND  0 DIFFERENTIAL EQUATIONS.  IW = 0.0.0.0.0.0.0.0.0.0.
THERE ARE  3 PARAMETERS WITH INITIAL ESTIMATES AND LIMITS AS FOLLOWS:

     NO.          INITIAL ESTIMATES      LOWER LIMITS      UPPER LIMITS

       1             160.355985          50.000000         200.000000
       2               1.04E500           0.500000           3.000000
       3               0.087430           0.000800           0.100000

ITER-
ATION PART  WEIGHTED SS          P 1           P 2           P 3          P
   1    1  0.167450E 04     0.160E 03.    0.105E 01     0.874E-01
1
CNO ERROR

   1    2  0.161195E 04     0.163E 03     0.104E 01     0.864E-01
   1    3  0.159137E 04     0.166E 03     0.104E 01     0.853E-01
   1    4  0.159136E 04     0.166E 03     0.104E 01     0.854E-01
   2    1  0.159136E 04     0.166E 03     0.104E 01     0.854E-01
1
CNO ERROR

   2    2  0.159133E 04     0.166E 03     0.104E 01     0.855E-01
   2    3  0.159134E 04     0.166E 03     0.104E 01     0.857E-01
   2    4  0.159133E 04     0.166E 03     0.104E 01     0.856E-01

                                BIOMATHEMATICS,UNIT 7292, UPJOHN

                        SUMMARY OF NON-LINEAR ESTIMATION

SZM000

AFTER  2 ITERATIONS THE ESTIMATES AND THEIR VARIABILITY ARE:

        NO.      ESTIMATE          STD. DEV.          95% CONFIDENCE LIMITS
          1   0.165730E 03     0.323925E 02     0.824638E 02     0.248997E 03 UNIVAR
                                                0.306761E 02     0.300784E 03 S PLANE

          2   0.103876E 01     0.393882E 00     0.262737E-01     0.205125E 01 UNIVAR
                                               -0.603447E 00     0.268097E 01 S PLANE

          3   0.855735E-01     0.364888E-01    -0.822264E-02     0.179370E 00 UNIVAR
                                               -0.665593E-01     0.237706E 00 S PLANE

TK1=     8.10TK2=      0.67
```

Figure 41. Iteration part of program 'NONLIN' during least-squares fit of plasma level data (Rat - 28) and best estimates of pharmacokinetic parameters following oral dose of ethosuximide (40 mg).

-- VARIANCE-COVARIANCE MATRIX OF ESTIMATES --

```
 0.32969E 01
-0.34342E-01    0.48746E-03
 0.32755E-02   -0.33938E-04    0.41834E-05
```

-- CORRELATION MATRIX OF ESTIMATES --

```
 0.10000E 01
-0.85665E 00    0.10000E 01
 0.88198E 00   -0.75153E 00    0.10000E 01
```

--EIGENVALUE ANALYSIS OF ESTIMATES--

EIGENVALUES	EIGENVECTORS		
0.2661E 01	-0.5949E 00	0.5653E 00	-0.5715E 00
0.2497E 00	0.6964E-01	0.7445E 00	0.6639E 00
0.8893E-01	0.8008E 00	0.3551E 00	-0.4823E 00

Figure 42. Eigenvalues and eigenvectors of the best estimates of pharmacokinetic parameters computed by program 'NONLIN' following oral dose of ethosuximide (40 mg) to Rat - 28.

FUNCTION 1

X	OBS. Y	CALC. Y	OBS-CALC	% DEVIATION	WEIGHT
0.83000E-01	0.35280E 02	0.13641E 02	0.21639E 02	61.34	0.10000E 01
0.25000E 00	0.62120E 02	0.37484E 02	0.24636E 02	39.66	0.10000E 01
0.50000E 00	0.64490E 02	0.65602E 02	-0.11119E 01	-1.72	0.10000E 01
0.10000E 01	0.81030E 02	0.10188E 03	-0.20859E 02	-25.73	0.10000E 01
0.20000E 01	0.13425E 03	0.12958E 03	0.46708E 01	3.48	0.10000E 01
0.40000E 01	0.12728E 03	0.12542E 03	0.18556E 01	1.46	0.10000E 01
0.80000E 01	0.96280E 02	0.91036E 02	0.52438E 01	5.45	0.10000E 01
0.12000E 02	0.59440E 02	0.64679E 02	-0.52391E 01	-8.81	0.10000E 01

SUM OF SQUARED OBSERVATIONS = 0.6285459E 05
SUM OF SQUARED DEVIATIONS = 0.1591333E 04
SUM OF WEIGHTED SQUARED DEVIATIONS = 0.1591333E 04
R-SQUARED = 0.975 COR= 0.940
S = 17.840269 WITH 5 D.F.

Figure 43. Observed and predicted values of plasma level by program 'NONLIN' according to two compartment open model following oral dose of ethosuximide (40 mg) to Rat - 28.

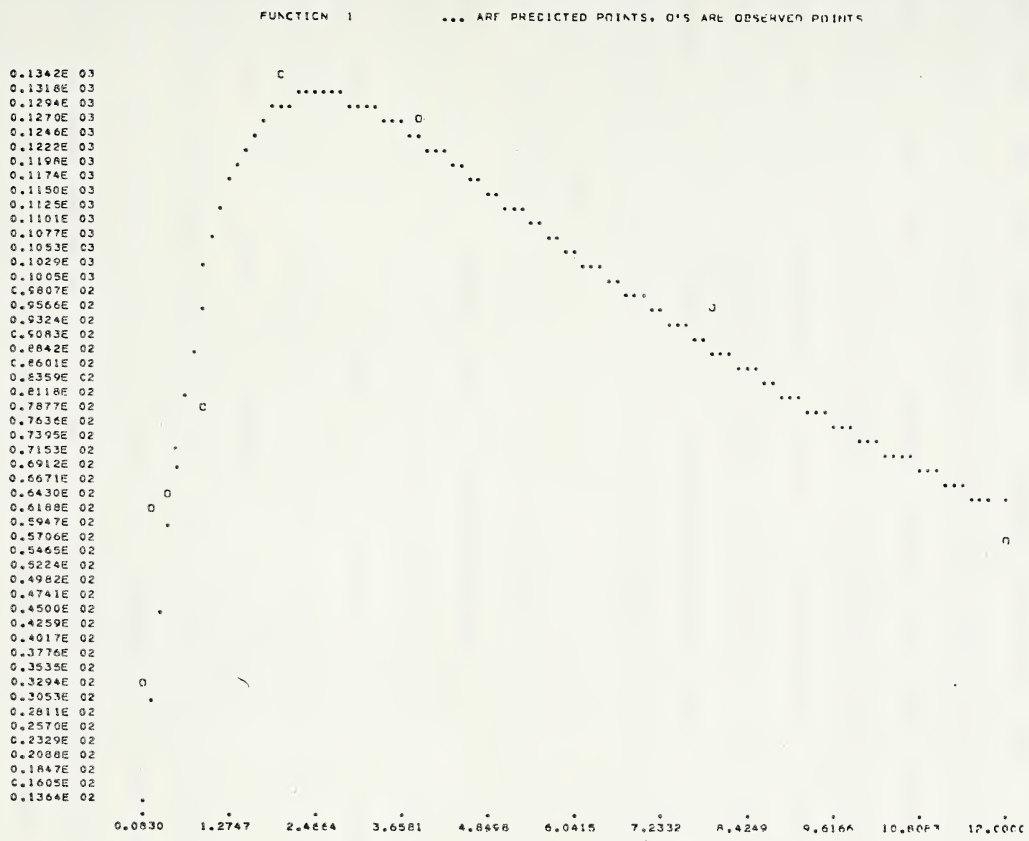

Figure 44. Least-square fit of plasma level data (Rat - 28) following oral administration of ethosuximide (40 mg) by program 'NONLIN'.

9. METABOLISM

a. In-Vitro Metabolism

Six male Wistar rats ranging in weight from 320 to 350 g were used in this experiment. Three rats were injected with 1 ml of phenobarbital sodium (12 mg/ml) for three consecutive days, a procedure known to promote enzyme induction in our laboratories (Coutts, personal communication). The other three rats served as controls. Each rat, in the two groups, was killed by decapitation, and the liver was quickly removed, freed from the gall bladder, washed in 1.15% ice-cold potassium chloride solution and blotted. The washed liver was weighed and homogenized with 4 volumes of ice-cold 1.15% potassium chloride solution in a stainless steel Waring blender. The homogenate was centrifuged at 9000 g for 30 minutes in a refrigerator centrifuge at 4°C. The supernatant was separated and 4 ml of the supernatant was used as source of enzyme.

The supernatant (4.0 ml) and 5 mcM NADP, 50 mcM glucose-6-phosphate, 25 mcM magnesium chloride and 100 mcM nicotinamide was placed in eight 100 ml beakers. 0.5 ml of a solution containing ethosuximide (1 mg) was added to four of the above eight beakers; 0.5 ml of phosphate buffer (pH 7.4) was added to the remaining four beakers which served as controls. The beakers were incubated aerobically at 37°C in Dubnoff metabolic incubator for 30 minutes. The contents of four control beakers were pooled and so also were the contents of four beakers containing ethosuximide. 8 ml of 20% zinc sulphate solution was added to the pooled incubated mixtures to precipitate the proteins. After five minutes of standing, 8 ml of a saturated solution of barium hydroxide was added drop-wise and shaken. The contents were transferred to

centrifuge tubes and centrifuged at 9000 g. The supernatant was extracted with chloroform under alkaline conditions (pH 11) followed by extraction of the aqueous layer with chloroform under acidic conditions (pH 2). The aqueous layers were finally neutralized with conc. ammonia and extracted with ethyl acetate. A portion of the supernatant obtained was also hydrolyzed for 30 minutes in a boiling water bath with 6 N hydrochloric acid and extracted with ethyl acetate.

The acidic, neutral, basic and possible hydrolisates of conjugated products were evaporated to 50 mcl and 2 mcl was injected into the gas chromatograph under the conditions described previously for analysis except that temperature programming at 4^0/min was also done from 100 - 250^0C.

The procedure was repeated with liver homogenates from phenobarbital treated rats.

The entire procedure was repeated for phensuximide and methsuximide.

b. In-Vivo Metabolism Study

Two hours following the oral administration of ethosuximide (40 mg) or methsuximide (40 mg) or phensuximide (40 mg) to three Wistar rats 10 ml of blood was collected, from each rat, by heart puncture into a heparinized syringe and centrifuged. The drug and the drug metabolites were extracted from 5 ml of plasma and analyzed in the manner described under in-vitro metabolism study. Blood samples from untreated animals served as controls.

Forty mg of ethosuximide was administered orally to 3 male Wistar rats weighing approximately 300 g. The rats were placed in individual

metabolism cages with water but no food. Urine was collected for 48 hours; pooled, and stored in a refrigerator until required for analysis. A portion of the urine was analyzed for free drug according to the analytical procedure described previously. Another portion of urine was hydrolyzed for one hour on a water bath in the presence of 6 N HCl and extracted with ethyl acetate and chromatographed in the usual manner.

The GLC method employed in this investigation to detect metabolites of ethosuximide did not reveal any peaks due to metabolites. Both an in-vivo and in-vitro metabolism study failed to show any metabolites of ethosuximide with the analytical procedure employed. It appeared that phenobarbital pre-treatment of the rat did not influence the in-vitro metabolism of ethosuximide using liver homogenate prepared from the treated animals. The GLC peak for ethosuximide in blood and urine extract or liver homogenate extract did not show any increase in the amount of ethosuximide following the acid hydrolysis of the blood, tissue or urine samples prior to the extraction procedure.

A metabolism study of methsuximide both in-vitro and in-vivo revealed presence of a metabolite peak with retention time of 8 minutes. Identification of metabolite by GLC-Mass Spectrometry was not successful due to on column absorption of the material.

Two peaks due to metabolites were observed in the plasma extract as well as in the liver homogenate extract of phensuximide. The retention times were 6.8 and 12.2 minutes respectively. Two similar peaks were also observed in a in-vitro metabolism study. Attempts to identify the metabolites by GLC-Mass Spectrometry failed due to on column absorption of the metabolite.

10. COMPARATIVE PHARMACOKINETIC STUDIES OF SUBSTITUTED SUCCINIMIDES

The plasma concentration time profile of ethosuximide, methsuximide or phensuximide was determined following intravenous injection into rats as described under blood level studies. The plasma level data was analyzed using program NONLIN (Metzler, 1968) as described above to determine the best estimates of the various pharmacokinetic parameters.

Structural similarity between a number of anticonvulsant drugs including ethosuximide, methsuximide and phensuximide have been shown in Figure 3 previously.

Plasma level data on ethosuximide following 40 mg I.V. dose to rats 4 - 9 is presented in Table A-2 and Table A-3. The plasma level data for methsuximide following 5.6 mg I.V. dose is presented in Table A-7. Table A-8 lists the plasma level data for phensuximide following 8.4 mg I.V. dose. Initial estimates of the various pharmacokinetic parameters according to a two compartment open model following I.V. dose (Figure 31) were determined graphically from the blood level data as described previously (Figures 16, 17 and 18). The best estimates of various pharmacokinetic parameters and derived rate constants were obtained from the analysis of blood level data according to a two compartment open model using digital computer least squares iterations as described earlier. Table XXVII lists the estimated pharmacokinetic parameters and derived rate constants for the three substituted succinimides.

TABLE XXVII

Pharmacokinetic parameters of substituted succinimides according to a two compartment open model following intravenous injection to Wistar rats

Parameters	Ethosuximide Dose = 40 mg N = 6	Methsuximide Dose = 5.6 mg N = 6	Phensuximide Dose = 8.4 mg N = 3
α hr^{-1}	5.77 + 2.2[a]	8.4 + 0.08	7.86 + 1.63
β hr^{-1}	0.076 + 0.068	0.257 + 0.093	0.321 + 0.034
K* hr^{-1}	0.119 + 0.085	0.982 + 0.25	0.554 + 0.052
$T_{\frac{1}{2}}$ hr	21.27 + 20.86	3.02 + 1.33	2.18 + 0.266
K_{ct}^{2} hr^{-1}	2.13 + 0.41	5.15 + 0.59	2.98 + 0.46
Ktc hr^{-1}	3.6 + 2.32	2.2 + 0.049	4.65 + 1.51
β/K	0.589 + 0.128	0.262 + 0.06	0.579 ± 0.13
Ktc/Kct	1.79 + 0.21	0.403 + 0.14	1.56 + 0.55
V_c % body weight	48.2 + 8.1	65.77 + 12.3	67.91 + 3.12
V_t % body weight	34.17 + 12.9	168.8 + 27.3	46.57 + 16.62

[a]Standard deviation.
*$\alpha.\beta/Ktc = K \neq \beta$

CHAPTER V

DISCUSSION

The success of any pharmacokinetic study depends on the availability of a sensitive, reproducible and rapid analytical procedure, which is specific for the drug under study. Gas liquid chromatographic (GLC) methods have been extensively used for the analysis of drug and/or their metabolites in biological samples, because of their simplicity, sensitivity, speed of analysis and reproducibility. A modification of the GLC method of analysis reported by Dill et al.(1965) was used for the quantitative determination of ethosuximide, methsuximide and phensuximide in blood, urine and tissue homogenates in this study. The method was relatively simple with high degree of accuracy and speed of analysis. The method was also specific for each of the drugs studied under the specified conditions of analysis. The method was capable of identifying and quantitating the three drugs simultaneously but this technique was not used in this study. The method was sensitive enough to determine as low as 0.1 mcg of drug per ml of a sample, although in practice the blood level analyzed was not less than 2 mcg/ml.

Among the seven stationary liquid phases screened to test their suitability for the analysis of succinimides, 3% OV-225 on chromosorb W (AW-DMCS) solid support was found to be the best. OV-1, OV-25, and OV-210, though used by some workers, were found to be poor with respect to peak resolution and the time required for an analysis. Greater degrees of tailing, peak broadening and long retention times were observed with these columns when compared with XE-60 or OV-225. Although the separation of the peaks on an XE-60 column was similar to that obtained on an OV-225 column some tailing of the peaks was observed with the former column. This interfered with the accurate

determination of the peak area. Therefore, OV-225 was selected and used in this study.

According to the plate theory of chromatographic separation, the degree of resolution of the individual components of a mixture is directly proportional to the number of theoretical plates present per unit length of the column. The number of theoretical plates calculated for a 3% OV-225 column at a linear gas velocity of 40 ml/min was 1555. This number is considerably smaller than 5000 plates recommended by Heftmann (1961) for efficient separation; however excellent peak resolutions were obtained for ethosuximide, methsuximide and phensuximide along with their respective internal standards under the conditions listed in Table I.

Flame ionization detection (FID) was chosen because of its greater sensitivity for detecting smaller amounts of the drug as compared with the thermal conductivity detector. It was found difficult to detect less than 100 mcg of the drug using a thermal conductivity detector (TC), but the peaks obtained with the use of TC detector were quite satisfactory for quantitation.

Using the column and the conditions established in Table I, the procedure was not only reproducible with the same instrument, but also was quite reproducible with different instruments. The retention times of the drug and its internal standard were reasonably short permitting the analysis of a large number of samples per day. On an average, following an extraction procedure, the time required for the analysis of one sample was less than seven minutes.

The peak area of the drug and internal standards was determined by triangulation and by an electronic integrator. A linear relationship was found between the peak area ratio (drug:internal standard) and the known amounts of the drug. The results of the triangulation method were quite satisfactory, but it was time consuming. It was also found difficult to accurately determine the peak area, if the detector response went off the recorder paper, which often happened with the unknown samples, since the attenuator settings, that would keep the detector response within the chart paper, were difficult to estimate accurately. Thus a particular analysis had to be repeated more than once which limited the number of analyses performed within a given time. The difficulties mentioned above were overcome by the use of an electronic integrator which accurately and reproducibly integrated the area under the peak even when the detector response went off the recorder paper. The electronic integrator printed out the peak areas along with the retention times for the peaks. The measurement was not only fast but also, with the retention times being printed out, there was no mistaking the identity of the peak being measured, from the extracts of the biological samples, amidst extraneous peaks which occasionally appeared. The result was processed almost simultaneously with the emergence of the peaks before the next injection was made.

α-α dimethyl - β -methyl succinimide has been used commonly as an internal standard for the GLC analyses of ethosuximide by a number of workers (Dill et al., 1965; Buchanan et al., 1969; Solow and Green, 1972). This chemical is expensive and not readily available. Kleijn et al. (1973) used naphthalene as an internal standard for the assay

of ethosuximide; but it was found unsuitable here, because of its different extraction properties in comparison with the drug. We screened several organic compounds in search of an internal standard and found biphenyl to be an excellent internal standard in terms of extraction properties and retention time. Therefore, biphenyl was used as the internal standard for the analysis of ethosuximide in this study. Phensuximide and methsuximide served interchangeably as the internal standard for the analysis of each other.

Chloroform was found to be a good solvent for the extraction of the drugs, under acid pH, from the biological samples. The extraction assembly permitted extraction of 40 samples at a time within 20 minutes. The absolute recovery of ethosuximide, methsuximide and phensuximide from various biological samples ranged between 96 - 107%.

Direct extraction of the drug from the tissues, blood or urine samples using chloroform as a solvent was very successful. No interfering peaks were found in the chloroform extracts of the blood, urine or tissue homogenates. Solow and Green (1971) converted ethosuximide to an N-methyl derivative in a flash heater before extraction; but the procedure seemed to be time consuming with no advantage in terms of peak resolution and analysis time and was not used.

The precision of the assay following the extraction procedure, expressed as a coefficient of the variation of the mean of 10 consecutive determinations, was found to be 2.02%. No deterioration of the drug was observed in the blood, urine or tissue homogenate samples stored at room temperature for 24 hours before analysis or following storage in a refrigerator for three weeks.

Calibration curves for ethosuximide, methsuximide and phensuximide did not change to any significant extent, when the calibration curves were repeated at later dates.

In general the GLC procedure for the analysis of these succinimides was found to be very efficient and reproducible.

* * * * * *

A limited blood level study of ethosuximide was reported by Hansen (1964), Dill et al. (1965) and Buchanan (1969). Blood level studies on phensuximide and methsuximide are relatively few. A methsuximide plasma level study following an oral administration of ^{14}C-methsuximide was reported by Nicholls and Orton (1972) and physiological disposition of phensuximide was reported by Glazko et al. (1954). No systematic pharmacokinetic study of succinimides could be found in the literature. Isolated reports were found following oral administration but no attempt was made to analyze the blood level data according to the pharmacokinetic concepts. Riegelman (1970) has pointed out that, in absence of intravenous blood level data, a significant over or under estimation of pharmacokinetic parameters usually results which can be misleading from a therapeutic standpoint.

In this study each one of the three succinimides showed a bi-exponential decay of the plasma level of the drug following intravenous administration. The fall in the plasma level of ethosuximide was very rapid for the first half hour followed by a very slow decrease in the plasma levels. The fall in plasma levels of methsuximide and phensuximide was also rapid within the first 40 minutes following the

intravenous dosing, followed by a relatively fast decrease in plasma level over the next three hour period. No change in the shapes of the plasma level curve was found for ethosuximide at the four dose levels studied. Since plasma levels of ethosuximide, methsuximide and phensuximide decreased biexponentially, the plasma level data was analyzed according to a two compartment open pharmacokinetic model scheme. A linear relationship was found between the zero time plasma concentration, following the intravenous dose of ethosuximide, and the administered dose, indicating the absence of dose dependent kinetics for ethosuximide, within the dose range studied. A wide intrasubject variation in biological half life was found in the rats similar to that reported in man (Wagner, 1973); however in most of the rats ethosuximide had a biological half life between the range 12 - 18 hours which is in agreement with the findings of Dill et al. (1965). The large variation in biological half life of ethosuximide in rats may be due to individual variations in the rates of metabolism of ethosuximide.

Table XIV lists the pharmacokinetic parameters of ethosuximide as a function of dose. The apparent volume of distribution did not change to any significant extent. A low value of β, the slow disposition rate constant was found at low dose level of 3 mg which cannot be explained. It may be due to inter-animal variation, or to some disease in the rats though the animals appeared healthy. The rate constant Ktc, is larger than Kct indicating a rapid return of the drug from the peripheral compartment to the central compartment for elimination. The ratio β/K, shows that over 50% of the drug in the body is available for elimination from the central compartment, yet the biological half life of the drug is long. This observation is an indirect indication of a very slow

rate of metabolism of ethosuximide or possible extensive tubular reabsorption of ethosuximide or both. The possibility of the long half life being due to extensive tubular reabsorption was examined in the urinary excretion study under the influence of changing urinary pH's and under the influence of inhibitors of tubular transport mechanisms, and will be discussed later.

Table XV shows the pharmacokinetic parameters of methsuximide and phensuximide. Considerably higher values of the rate constant Kct were found for methsuximide in comparison with phensuximide indicating the greater tendency of methsuximide to partition into the peripheral compartment, which is consistent with the high lipid solubility characteristics of the drug (Nicholls and Orton, 1972).

Though the elimination rate constant for phensuximide is smaller than the elimination rate constant for methsuximide, the biological half life of methsuximide was found to be slightly longer than the biological half life of phensuximide. This observation may be explained in view of the fact that a greater percentage of phensuximide (almost double) is available in the central compartment for elimination than methsuximide as seen from the value of rate constant Ktc, and the ratio, β/K. Ethosuximide, methsuximide and phensuximide appear to be fairly well distributed all over the body as seen from the value of the apparent volume of the central compartment which was over 50% of the body weight. The intravenous studies indicated that the pharmacokinetics of the succinimides can be accounted for by using a two compartment pharmacokinetic model.

Ethosuximide was found to be absorbed rapidly following the oral dose of the solution to the rats. There was very little difference

in the area under the plasma level time curve following intravenous or oral dosing with ethosuximide. This indicated almost complete absorption of the drug from the GIT of the rat with an insignificant first pass effect. The maximum blood level was reached within two hours following the oral dosing of ethosuximide to rats and was similar to that reported in man (Buchanan et al., 1969). The variations in the value of the absorption rate constant, Ka, found in the rats were comparable with those reported in man (Buchanan et al., 1969). The fraction of the dose absorbed per volume of distribution, FD/V, in the rat, was 2 - 3 times greater than that reported for man (Buchanan et al., 1969). This difference appears to be due to the difference in the dose (mg/kg) administered to the rats (dose in man, 10 mg/kg; in rat, 80 mg/kg), and possible differences in the volume of distribution. The reported biological half life of ethosuximide in man was 2 - 3 times greater than that reported in the rat, which may be indicative of the greater capacity of rats to metabolize ethosuximide than man.

The oral blood level study indicated that the pharmacokinetics of ethosuximide in rat were quite similar to man except in terms of metabolism rates. Therefore, with certain necessary correction factors, the data from rats may be projected to man; or rats could be used as a model animal for the routine quality control of ethosuximide formulations.

Knowledge about the effect of successive doses on the plasma levels following a multiple dosing of ethosuximide may be quite valuable in therapeutic management of a patient in terms of dose scheduling. Pharmacokinetic findings from a single dose blood level data have been used to predict the plasma levels of drugs during chronic administration

(Wagner and Metzler, (1969). Very little information was available on the plasma levels of ethosuximide in man or animals following a multiple dose regimen. On the basis of limited blood level data, following a multiple oral dosing of ethosuximide in man (Haerer et al., 1970) it was difficult to test the validity of the dose regimen used in the clinical practice. Therefore a multiple dose level study in rat was undertaken to compare the theoretically predicted blood levels with the experimentally observed blood levels. The dosing interval chosen was 12 hours which was found to be the half life of ethosuximide in a number of rats. It was hoped that this dosing schedule would not lead to excessive drug accumulation, and yet offer significant information to evaluate a pharmacokinetic model that may accurately predict the plasma levels during a multiple dose regimen. The plasma level was found to reach a plateau after the fourth dose (48 hours). The plasma levels during the steady state ranged, in three rats, between 60 - 90 mcg/ml. Haerer et al. (1970) have reported 40 mcg/ml blood concentration of ethosuximide in man during chronic administration. Following the discontinuation of the dose, the plasma level declined monoexponentially as in a single dose with an insignificant change in the half life. This finding indicated that ethosuximide was not likely to induce its own metabolism during the multiple dosing. Wagner and Metzler (1969) have indicated that, for most practical purposes, a one compartment pharmacokinetic model could be used without significant error for predicting the plasma levels during multiple dosing. An attempt was therefore made to predict the plasma levels of ethosuximide in rats following a multiple dosing schedule on the basis of the pharmacokinetic information from a single dose blood level study

using a 'two' and a two compartment pharmacokinetic models. Multiple dose calculations were made with the help of the computer program reported by Niebergall et al. (1974). Both a 'two' and a two compartment model were found to predict much higher blood levels of ethosuximide in rat than was observed experimentally after multiple oral dosing (the value of K in the two compartment model was assumed to be the same as β). The predicted values according to a 'two' and a two compartment models with $K = \beta$, were very close, as was shown by Wagner and Metzler (1969); but according to equation 17, $K = \frac{\alpha\beta}{Ktc}$ in a two compartment open model. Therefore it was felt that K could not be equal to β and the differences found between the observed and predicted plasma levels may be explained in terms of the actual values of α, β and Ktc from a single dose intravenous study. Indeed it was found that the two compartment model using the rate constants α, β and Ktc predicted plasma levels which were very close to the observed plasma levels.

If one assumes the real therapeutic plasma levels to be 70 mcg/ml, in Figure 21, which could be achieved experimentally with 40 mg ethosuximide given every 12 hours, the predicted blood levels according to a'two'compartment open model would be above 150 mcg/ml. The experimenter might thus be tempted to reduce the dose, which may actually result in subtherapeutic blood levels. The results of this study indicate that plasma level predictions according to a 'two' compartment open model for ethosuximide, in absence of the pharmacokinetic parameters from intravenous blood level data may be dangerously misleading. The blood level data reported by Haerer et al. (1970) in one of their subjects, was compared with the predicted blood levels according to a 'two' compartment open model using the pharmacokinetic information from the single dose

blood level data (Buchanan et al., 1969). Figure 22 shows the extremely large difference between the observed and predicted blood levels according to a one compartment open model in subject 1. Figure 22 clearly shows that, while the observed plasma level of ethosuximide in man following multiple dose regimen (250 mg t.i.d. oral) for two months was 40 mcg/ml, the predicted blood level according to a 'two' compartment model, was close to 300 mcg/ml.

Since the values of Ka, the absorption rate constant and FD/V, the fraction of the dose absorbed per volume of distribution in rat and man were found to be comparable, it was reasonable to assume that the disposition rate constants Kct and Ktc in man may be similar to that in rat. Therefore using the Kct and Ktc values from the rats and the values of Ka and FD/V from man, the plasma level was predicted in subject 1, according to a two compartment pharmacokinetic model. The value of the rate constant β, was the reported value of K in man according to a'two'compartment open model (Buchanan et al., 1969). The predicted values according to a two compartment open model closely agreed with the observed blood level in subject 1. A comparative look at the predicted points according to a'two'compartment open model and the observed points in man and rat (Figures 21 and 22) may lead one to suspect that ethosuximide on multiple dosing must have induced its own metabolism which was responsible for the observed low plasma level. However the fact that the half life of ethosuximide did not change on multiple dosing in the rat suggests that such an interpretation is not possible. The importance of the intravenous data is evident for the prediction of blood level following multiple dosing. With the

actual plasma level data from multiple dosing and the plasma level data following the discontinuation of the dose it may be possible to calculate Ktc and Kct values using digital computer iterations. It appears however that one needs a two compartment open model to accurately predict plasma concentrations of ethosuximide in rat and man, during a multiple dose regimen.

* * * * * *

Certain pharmaceutical adjuvants affect the systemic availability of some drugs because of physical or physico-chemical interactions between the drug and the adjuvants. It was reported that when the adjuvant calcium sulphate was replaced by lactose, in the formulation of diphenylhydantoin capsule, an excessive increase in bioavailability was found, which resulted in severe toxicity (Tyrer et al., 1970). It has been proposed that diphenylhydantoin formed a complex with the calcium sulphate diluent, making it less available; and hence the dose schedule followed with the older formulation was safe; however without any interaction between lactose and diphenylhydantoin the whole of the dose in the formulation was absorbed leading to the observed toxic effects following the old dose schedule with the new formulation. It is also known that certain OTC products like antacids, when taken concomitantly with tetracyclines, affect the systemic availability of tetracycline due to interference with the absorption process. Since no information was available on the bioavailability of ethosuximide in the presence of some pharmaceutical adjuvants and antacids, the current study was undertaken. Mean values of various pharmacokinetic

parameters calculated, according to a 'two' compartment pharmacokinetic model, from the blood level data of each formulation, are tabulated in Table XXI.

The maximum plasma concentration (C_{max}) achieved with each formulation does not vary to any significant extent; however C_{max} values were considerably lower for ethosuximide in the presence of olive oil and calcium chloride. Significant reductions in the fraction of the dose absorbed per volume of distribution, FD/V was found in the presence of calcium chloride, calcium carbonate, olive oil and activated charcoal. A considerable reduction in the value of the absorption rate constant, Ka, was found in formulation containing olive oil, or calcium chloride and in the Zarontin^{R} Capsule contents. A shift in the T_{max}, the time to reach maximal plasma concentration, following the oral dosing of the rats was observed for the Zarontin capsule formulation (capsule contents), the ethosuximide formulation containing calcium chloride, and the ethosuximide formulation in the presence of activated charcoal. Approximately 3 hours lag time was observed in the absorption of ethosuximide for the above mentioned formulations. Plasma half lives for ethosuximide in the presence of methylcellulose, activated charcoal, olive oil and calcium carbonate could not be estimated accurately within the time period of this study (12 hours), because of the influence of continued absorption on the elimination curve. Some variations in half life were observed with some formulations, but it could not be the effect of formulation, since the formulation variables do not influence the elimination processes. The variation may be due to wide inter-subject variation among the rats. The wide inter-subject

variation of biological half lives in man supports this explanation (Wagner, 1973b), that the variation in $T_{\frac{1}{2}}$ observed in some formulations could not be attributed to formulation variables.

A significant reduction in the value of the area under the plasma level time curve, AUC (a measure of the extent of absorption or systemic availability), was found for the ethosuximide formulations containing activated charcoal, calcium carbonate, calcium chloride or magnesium trisilicate. The percent systemic availability of ethosuximide for the formulations studied is shown in Figure 24. A statistically significant decrease in the systemic availability of ethosuximide occurred in the presence of a soluble calcium chloride salt and in the presence of calcium carbonate which may become soluble in the acid gastric medium; but insoluble tribasic calcium phosphate did not influence the systemic availability of ethosuximide. Magnesium trisilicate, an antacid containing a divalent metal was also found to reduce the availability of ethosuximide; but the antacid aluminum hydroxide gel, which has a trivalent metal present did not affect the percent systemic availability of ethosuximide. The influence of the soluble calcium salts and magnesium trisilicate on the availability of ethosuximide cannot be explained with certainty; but one may speculate that some weak complex between the soluble calcium and magnesium ions and ethosuximide may be responsible for the observed influence. Subtle differences in the covalent, atomic and ionic radii (Ca: 1.74, 1.97, 0.99; Mg: 1.3, 1.6, 0.65; Al: 1.18, 1.43, 0.5 A^{o}) of calcium, magnesium and aluminum may be responsible for the observed influence of these ions on the systemic availability of ethosuximide.

In view of the small number of subjects used in this study, no definite conclusions can be drawn regarding the influence of the antacids magnesium trisilicate or calcium carbonate, on the systemic availability of ethosuximide; however the study does indicate the following facts: 1) The concomitant administration of magnesium trisilicate or calcium carbonate containing antacids with ethosuximide may pose some possible drug interaction problems in terms of the drug bioavailability; 2) Although activated charcoal reduced the extent of the absorption of ethosuximide, its value in the toxicological treatment of poisoning from ethosuximide is doubtful, in view of the rapid absorption of ethosuximide; 3) Calcium triphosphate may be a possible adjuvant for the solid dosage formulation of ethosuximide; and 4) The commercial capsule and syrup formulations are equally available.

* * * * *

In view of the fact that ethosuximide is negligibly protein bound, is very little metabolized, has a long half life of 60 hours in man, 22 hours in monkey and 12 hours in rats, and is excreted unchanged in the urine to the extent of 13 - 19% (Chang et al., 1972), a study of the urinary excretion kinetics of ethosuximide was undertaken in an attempt to understand the cause of the long half life of the drug. It is recognized that pH dependent urinary excretion occurs for many dissociable drugs of pKa between 3.0 - 7.5 for acid drugs and between pKa 7.5 - 10.5 for basic drugs (Milne, 1965; Orloff and Berliner, 1956; Astatoor et al., 1965; Beckett et al., 1965).

The results of the urinary excretion of ethosuximide under the uncontrolled, the alakaline and the acidic urinary pH's and under the influence of probenecid, an inhibitor of tubular transport mechanism,

are shown in Table XXV. The values of elimination rate constants K, determined from the urinary excretion rate versus time plot and from the plot of the percent of unchanged ethosuximide remaining to be excreted versus time were in close agreement with the value found from the blood level versus time plot. The cumulative amount of the unchanged ethosuximide excreted in the urine was neither influenced by the changes in the urinary pH under the acid or the alkaline treatment, nor by the probenecid treatment. A significant reduction in maximum urinary excretion rate of ethosuximide was found in rats pretreated with ammonium chloride (urine pH 5 - 5.5). The peak excretion time of ethosuximide varied considerably under the various pretreatment conditions. A lag time (peak excretion rate time - peak plasma concentration time of most available formulation) of about four hours was found under the acid and the uncontrolled urine pH, and a lag time of about four hours was observed in the absorption of ethosuximide under the probenecid treatment. The urinary excretion rate constants, Ku, computed from the y intercept, of the semilogarithmic plot of excretion rate versus time plot, and that computed from the relationship: $Ku = (De)_{\infty}.K/FD$, were similar. A very low value for the excretion rate constant, Ku was observed under all the treatments. A significant reduction in the value of urinary excretion rate constant, Ku, was also found in animals pretreated with sodium bicarbonate and ammonium chloride. In light of the pH-partition hypothesis it is difficult to explain the observations cited above because between pH 5 - 8, ethosuximide would be virtually unionized (94 - 99.9%). It is more likely that the urinary excretion profile of ethosuximide could not be influenced by changing urinary pH. A very

low clearance value of 2.4 ml/hr was also found for ethosuximide. The percent of the dose excreted unchanged under the four treatments studied did not differ significantly at 95% level of confidence according to the analysis of variance for a complete randomized design, and according to the student 't' test. Only 10 - 17% of the administered dose of ethosuximide was excreted unchanged in the urine which is in agreement with the reported value of 13 - 19% excretion in man, monkey and rats (Chang et al., 1972). Therefore the low clearance value for ethosuximide could be attributed to extensive tubular reabsorption. Since probenecid, the tubular transport inhibitor did not have any influence on the percent of unchanged drug excreted in the urine, tubular reabsorption must be a passive process. The apparent significantly longer biological half life of ethosuximide under the alkaline and the acid urinary pH conditions was within the range of the individual biological half life variations. This study indicated that the urinary excretion of unchanged ethosuximide can neither be influenced by changes in urinary pH, nor by treatment with probenecid, and that the long half life of the drug is attributable to slow metabolism and extensive passive tubular reabsorption.

Ethosuximide was reported to be uniformly distributed throughout the body following its administration to animals (Dill et al., 1965). The pharmacokinetic analysis of the plasma data done in this laboratory supports this statement of Dill et al. (1965). Following an intravenous dose of ethosuximide to rats some tissues were analyzed for ethosuximide content to confirm the uniformity of the tissue distribution of ethosuximide. It was found that ethosuximide was uniformly distributed in

the rat tissues. The concentrations of ethosuximide in the various tissues were quite comparable to the plasma concentrations at the various sampling times. Some variations observed in the tissue concentrations of ethosuximide may be due to the adhering body fluid which may have contributed additional amounts of ethosuximide to the tissues analyzed. The tissues were not washed for fear of washing out some of the ethosuximide. The tissue levels obtained were found to be quite a bit higher than those reported by Chang et al. (1972). This difference could be attributed to the different routes of administration and dose levels. The tissue distribution study reported by Chang et al. (1972) was following the oral administration of ethosuximide; whereas the study reported in this work was following an intravenous administration. The concentration of the drug in the whole kidney was similar to the concentration in the kidney cortex, indicating probable absence of an active reabsorption process. The level of ethosuximide in the fat tissue was also comparable to the ethosuximide levels in the other tissues analyzed, which contradicts the report of Chang et al. (1972). The volume of distribution of ethosuximide found from the pharmacokinetic analysis of blood level data was over 50% of the body weight, which further supported the view that ethosuximide was uniformly distributed throughout the body.

Four pharmacokinetic models were tested for their suitability to fit the observed plasma level data, of ethosuximide, methsuximide and phensuximide, from rats. The plasma level data following an intravenous dosing was best described by a two compartment pharmacokinetic model. The computer fit to the plasma level data according to a one compartment

open model was very poor. No improvement in the quality of the fit was found with the use of a three compartment pharmacokinetic model.

The oral plasma level data of ethosuximide was best described by a 'two' compartment model according to the scheme shown in Figure 32. A two compartment pharmacokinetic model scheme (Figure 33) did not improve the quality of the fit. The study confirmed the validity of a two compartment open model for the analysis of the plasma level data following the intravenous administration of ethosuximide, and a 'two' compartment model following a single oral dose of ethosuximide; however, the prediction of the plasma level during a multiple dosing schedule was much more accurate when a two compartment pharmacokinetic model was used.

Iterative least square fitting of the plasma level curve with an appropriate Digital Computer Program was the most accurate and fast way for the estimation of various pharmacokinetic parameters and for the testing of the validity of the proposed pharmacokinetic models. Over or under estimations of pharmacokinetic parameters by graphical techniques have been reported by Wagner and Metzler (1967). The authors reported that the elimination rate constant values obtained graphically were 12 - 13% lower than the real values, whereas the absorption rate constant values estimated graphically were 13 - 27% higher than the true values.

Program 'NONLIN' (Metzler, 1968) was found adequate for the iterative least squares fitting of all blood level data in this study. Once the initial problem of adapting the program to the computer system of the University of Alberta in Edmonton was solved, and a little experience was gained in the preparation of the data deck with the

subroutine 'DFUNC', the program was found most efficient and simple to fit the plasma level data and to calculate the derived pharmacokinetic parameters and to test the validity of the proposed pharmacokinetic models.

The CPU time required to process four sets of the blood level data according to a two compartment pharmacokinetic model was less than ten seconds. Over 30 pages were printed with the necessary iterations, the statistical analysis, the parameter estimations and the curve fitting within ten seconds. The approximate cost for such a run was $1.50. Without the help of digital computer program this work could never have been completed within a reasonable time.

Notari (1973) has reviewed the methods for assessing structural effects on pharmacokinetic parameters using literature data for penicillin derivatives. The pharmacokinetic parameters, and hence the biological response, of some drugs can be modified by subtle structural changes. Screening processes in the drug design may be optimized through the pharmacokinetic studies on structurally related analogs.

Ethosuximide, methsuximide and phensuximide are closely related derivatives of succinimide. Their structural differences and structural relationships to other anticonvulsants have been discussed previously.

The major differences in the pharmacokinetic parameters of ethosuximide, methsuximide and phensuximide were found with respect to the slow disposition rate constant β, and the overall elimination rate constant K. The values of β for methsuximide and phensuximide were found to be 3 - 4 times greater than the value of β for ethosuximide. Therefore a better control of petit mal epilepsy with ethosuximide may be attributed to the longer half life of ethosuximide compared to the

half lives of phenyl substituted succinimides. The overall elimination rate constant for methsuximide was 8 times greater than the overall elimination rate constant for ethosuximide; whereas the overall elimination rate constant for phensuximide was about 5 times greater compared to the elimination rate constant for ethosuximide. This information indicated that a relatively larger dose of the phenyl-substituted succinimides may be required for the control of petit mal epilepsy compared with the dose of ethosuximide. This speculation from the pharmacokinetic data is in agreement with the clinical reports according to which higher doses of phensuximide are required for the control of the petit mal epilepsy when compared with the response of ethosuximide in man. The ratio β/K indicated that 58% of ethosuximide and phensuximide in the body and 26% of methsuximide in the body are available in the central compartment at any given time for elimination, yet the biological half lives of phensuximide and methsuximide were found to be shorter than the biological half life of ethosuximide, indicating a comparatively slow rate of metabolism for ethosuximide. The comparison of the values of Kct indicated a tendency of methsuximide to favour the peripheral compartment, which may be the reason for the slightly greater half life of methsuximide compared with the half life of phensuximide. Each of the three succinimides appeared to be well distributed throughout the body, based on their apparent volumes of distribution of central compartment which was above 50% of the body weight.

No metabolite of ethosuximide could be detected with the procedures employed in this study. It was tempting to conclude that metabolism of ethosuximide is negligible, in support of the earlier reports

(Dill et al., 1965); however it was realized than an extensive analytical methodology has to be employed to conclude with certainty that ethosuximide is not metabolized. Further it may be necessary to attempt some derivatization of possible polar metabolites before GLC analysis. Therefore, the metabolism study was set aside until the complete pharmacokinetics of the unchanged drug were completed. During this period Horning et al. (1973a) reported the isolation and identification of four monohydroxy metabolites of ethosuximide in the urine of rat and man. Several glucuronides of monohydroxyethosuximides have also been found by these authors using a derivatization technique and other involved separatative techniques. Thus the long biological half life of ethosuximide may be due to extremely slow rates of metabolism. This is also suggested from the pharmacokinetic parameters of ethosuximide. Methsuximide and phensuximide are known to undergo rapid metabolism. Parahydroxylation of the phenyl substituent at the α-carbon and N-demethylation of methsuximide are the major routes of metabolism (Nicholls, 1972; Dudley et al., 1972). In-vitro and in-vivo metabolism studies indicated two metabolites of methsuximide and one metabolite of phensuximide, though we could not, positively, identify the metabolites due to on column absorption of the metabolites in the GLC-Mass Spectrometer. The column that we used regularly in the analysis of blood levels of methsuximide and phensuximide was usually saturated with the metabolites, therefore the metabolite peaks could be seen on the regular trace; but the regular column used in the routine analysis did not fit the GLC-Mass Spectrometer unit. The unchanged drug, being in higher concentration than the metabolites, were easily identified as parent drug by GLC-Mass

Spectrometry. The spectra obtained from the plasma extracts of the animals previously treated with the drug, were identical with the parent drug spectrum and with the published spectrum (Locock and Coutts, (1970)).

CHAPTER VI

SUMMARY AND CONCLUSIONS

The following conclusions and recommendations for further study can be made from the results of this study which was the basis of this thesis.

Conclusions

1. A gas-liquid chromatographic procedure was developed for the analysis of ethosuximide, methsuximide and phensuximide in blood, urine or tissue homogenates of rats.
2. The method developed was accurate, sensitive, reproducible and rapid.
3. Ethosuximide was not bound to plasma proteins.
4. A two compartment pharmacokinetic model was found to accurately describe the plasma levels of the three drugs following an intravenous injection.
5. A 'pseudo' one compartment pharmacokinetic model described the plasma level of ethosuximide following oral dosing.
6. The absorption of ethosuximide from the GIT of rats was rapid and complete. There were marked individual variations in the absorption rate constant of the drug. The absorption rate constant was found to be approximately 3.0/hr which was similar to that reported in man.
7. The elimination rate constant for ethosuximide in rat was about three times larger than that reported in man.
8. Ethosuximide did not show dose dependent kinetics within the dose range of 3 mg to 40 mg (8.0 - 120 mg/kg).

9. The absorption of ethosuximide from GIT of rat was reduced in presence of olive oil, activated charcoal and calcium and magnesium ions.
10. The systemic availability of ethosuximide from Zarontin syrup, Zarontin capsule and ethosuximide solution dose was similar to the availability from an intravenous dose, however the systemic availability (over 12 hours) of the drug was significantly reduced in the presence of soluble calcium and magnesium ions and in the presence of activated charcoal and olive oil.
11. The use of activated charcoal in the treatment of acute poisoning due to ethosuximide may not be successful because of the extremely fast absorption rate of ethosuximide.
12. Elimination of ethosuximide cannot be influenced by control of urinary pH within the range 5.0 - 8.3 or by treatment with an inhibitor of tubular transport mechanisms.
13. The long biological half life of ethosuximide in rat appeared to be due to extensive passive tubular reabsorption, and a relatively slow rate of metabolism.
14. Plasma levels of ethosuximide during multiple dose regimens can be accurately predicted both in rat and man by computers using a two compartment pharmacokinetic model.
15. The observed differences in the pharmacokinetics of ethosuximide, methsuximide and phensuximide in rats can be explained in terms of differences in their rates of metabolism and rates at which the drugs return back to the central compartment from the peripheral compartment for elimination.

16. Methsuximide and phensuximide are metabolized faster than ethosuximide by the rats.
17. The slightly longer half life of methsuximide compared to phensuximide may be explained in terms of a faster return of phensuximide from the peripheral compartment to the central compartment The return of methsuximide being affected by its greater affinity for the peripheral compartment than ethosuximide.

Recommendations for Further Study

1. Systematic study of the partitioning behaviour of ethosuximide, methsuximide and phensuximide as a function of pH.
2. Study of the influence of other anticonvulsants on the pharmacokinetics of ethosuximide.
3. Influence of the coadministration of ethosuximide, methsuximide and phensuximide on the pharmacokinetic properties of each drug during a multiple dose regimen.
4. Study of the pharmacokinetics of the metabolites of the three drugs.

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APPENDIX A

TABLE A-1

Blood levels following I.V. administration of 40 mg of ethosuximide to male Wistar rats

Time (minutes)	Blood levels Rat - 1 (372 g)* mcg/ml	Rat - 2 (410 g) mcg/ml	Rat - 3 (380 g) mcg/ml
2.0	173.1	218.4	183.6
3.0	168.5	208.2	172.5
4.0	166.5	204.8	160.6
5.0	155.5	192.5	155.2
10.0	154.8	172.5	140.3
20.0	134.9	139.8	118.2
40.0	125.2	112.8	108.5
60.0	118.2	98.8	97.2
90.0	115.7	95.5	95.4
120.0	107.8	92.2	93.2
240.0	98.1	84.0	86.0
360.0	90.1	82.0	78.0
540.0	76.1	74.0	68.0
720.0	64.0	66.0	58.0

*Weight of the rat in grams.

TABLE A-2

Blood level following I.V. administration of 40 mg of ethosuximide solution to male Wistar rats

Time (minutes)	Blood levels Rat - 4 (375 g)* mcg/ml	Rat - 5 (400 g) mcg/ml	Rat - 6 (400 g) mcg/ml
1.0	155.54	-	-
2.0	243.4	218.4	249.2
3.0	172.98	218.2	243.0
4.0	168.5	209.7	254.7
5.0	166.5	152.5	211.5
7.0	174.9	169.1	231.7
10.0	154.7	186.6	168.9
20.0	134.8	147.0	145.7
40.0	129.9	112.8	143.7
60.0	125.4	98.9	104.3
90.0	115.7	124.9	136.7
120.0	107.8	106.7	133.0

*Weight of the rat in grams.

TABLE A-3

Blood level following I.V. administration of 40 mg of ethosuximide solution to male Wistar rats

Time (minutes)	Blood levels Rat - 7 (365 g)* mcg/ml	Rat - 8 (400 g) mcg/ml	Rat - 9 (388 g) mcg/ml
1.0	-	163.9	145.5
2.0	244.9	-	-
3.0	222.2	153.3	183.6
4.0	204.4	148.9	160.6
5.0	234.9	159.0	122.7
7.0	188.7	146.0	147.1
10.0	210.4	139.1	144.3
20.0	153.3	113.5	101.0
40.0	169.9	-	104.9
60.0	295.4	131.3	97.0
80.0	108.5	107.9	102.3
90.0	-	-	-
100.0	127.9	105.8	100.7
120.0	-	75.9	89.8

*Weight of the rat in grams.

TABLE A-4

Blood level following I.V. administration of 10 mg of ethosuximide solution to male Wistar rats

Time (minutes)	Blood levels Rat - 10 (284 g)* mcg/ml	Rat - 11 (284 g) mcg/ml	Rat - 12 (280 g) mcg/ml	Rat - 13 (282 g) mcg/ml
2.0	54.6	-	-	-
2.5	-	54.8	52.4	53.1
4.0	67.5	-	-	-
4.5	-	55.9	49.2	52.2
7.0	46.3	51.4	55.3	48.5
9.0	-	70.6	40.1	51.7
11.0	42.2	-	-	-
12.0	-	45.2	44.1	37.9
20.0	36.1	-	-	-
22.0	-	43.0	42.9	39.3
30.0	37.0	-	-	-
32.0	-	43.0	37.6	39.3
41.0	35.1	-	-	-
50.0	35.3	-	-	-
52.0	-	36.7	40.4	42.8
70.0	31.7	-	-	-
72.0	-	33.4	32.8	39.7
102.0	-	-	33.9	31.9
105.0	37.5	-	-	-
120.0	-	-	36.8	34.4
140.0	33.5	-	-	-
160.0	-	-	40.2	40.7
180.0	31.2	-	36.1	31.5

*Weight of the rat in grams.

TABLE A-5

Blood level following I.V. administration of 5.0 mg of ethosuximide solution to male Wistar rats

Time (minutes)	Blood levels Rat - 14 (344 g)* mcg/ml	Rat - 15 (352 g) mcg/ml	Rat - 16 (272 g) mcg/ml	Rat - 17 (316 g) mcg/ml
5.0	19.0	28.0	36.6	31.4
9.0	18.8	21.6	35.2	25.8
12.0	15.8	21.1	26.8	19.3
15.0	16.1	20.7	26.6	18.4
30.0	15.4	18.4	24.0	27.8
60.0	15.1	19.6	17.5	14.4
120.0	14.0	12.9	17.9	16.8
180.0	14.7	14.1	19.9	19.1
240.0	13.5	12.6	20.8	19.1
300.0	14.4	11.8	21.5	16.9
540.0	11.2	12.26	14.7	15.6

*Weight of the rat in grams.

TABLE A-5 (continued)

Blood level following I.V. administration of 5.0 mg of ethosuximide solution to male Wistar rats

Time (minutes)	Blood levels Rat - 18 (445 g)* mcg/ml	Rat - 19 (500 g) mcg/ml	Rat - 20 (460 g) mcg/ml	Rat - 21 (500 g) mcg/ml
1.0	20.6	-	-	-
2.0	-	16.96	-	17.48
3.0	19.0	-	-	-
6.0	-	13.9	-	14.8
9.0	14.8	10.8	19.8	16.2
16.0	15.0	11.2	11.2	13.3
25.0	9.3	10.2	12.3	10.1
45.0	10.0	8.5	11.5	10.8
65.0	10.3	8.4	10.4	10.6
85.0	-	8.6	10.6	-
105.0	9.3	-	-	11.5
125.0	10.3	8.9	7.6	9.7
145.0	9.4	9.4	9.9	9.9
165.0	9.4	9.4	10.3	10.7
185.0	10.9	8.7	10.6	9.1
205.0	-	10.7	12.5	10.9

*Weight of the rat in grams

-Sample either not collected or lost.

TABLE A-6

Blood level following I.V. administration of 3.0 mg of ethosuximide solution to male Wistar rats

Time (minutes)	Blood levels Rat - 22 (392 g)* mcg/ml	Rat - 23 (448 g) mcg/ml	Rat - 24 (460 g) mcg/ml	Rat - 25 (420 g) mcg/ml
5.0	14.6	12.7	10.0	16.6
7.0	-	9.8	-	-
10.0	9.9	-	-	8.4
15.0	7.2	8.2	-	12.3
22.0	8.4	-	8.0	10.9
42.0	8.1	7.5	6.4	14.6
62.0	8.4	7.6	6.8	7.8
82.0	7.7	7.7	6.6	6.8
102.0	7.9	6.6	6.2	9.2
122.0	6.9	6.5	7.3	19.7[C]
142.0	6.5	6.5	6.7	16.4[C]
182.0	9.2	6.9	6.0	23.04[C]
242.0	8.1	6.6	6.3	18.6[C]

*Weight of the rat in grams.

[C]Cross contamination from syringe.

TABLE A-7

Blood level following I.V. administration of 5.6 mg of methsuximide solution to male Wistar rats

Time (minutes)	Blood levels Rat - 26 (400 g)* mcg/ml	Rat - 27 (412 g) mcg/ml	Rat - 28 (319 g) mcg/ml
3.0	12.94	18.5	16.8
6.0	9.95	13.12	11.61
12.0	7.11	8.11	7.6
24.0	5.2	6.6	4.5
48.0	4.0	4.2	3.7
96.0	3.1	3.2	2.8
192.0	2.0	2.1	1.8

*Weight of the rat in grams.

TABLE A-8

Blood level following I.V. administration of 8.4 mg of phensuximide solution to male Wistar rats

Time (minutes)	Blood levels Rat - 29 (350 g)* mcg/ml	Rat - 30 (380 g) mcg/ml	Rat - 31 (500 g) mcg/ml
3.0	29.13	26.2	21.5
6.0	25.8	25.3	19.1
12.0	19.8	19.8	16.8
24.0	16.6	16.9	14.9
48.0	12.8	12.7	11.9
60.0	12.4	12.5	11.5
96.0	11.1	10.9	10.8
116.0	9.4	9.5	9.4

*Weight of the rat in grams.

TABLE A-9

Blood level following oral administration of 40 mg of ethosuximide solution to male Wistar rats.

Time (minutes)	Blood levels Rat - 32 (416 g)* mcg/ml	Rat - 33 (415 g) mcg/ml	Rat - 34 (416 g) mcg/ml
5.0	35.28	33.15	30.6
15.0	62.12	41.69	48.3
30.0	64.49	55.78	59.35
60.0	81.03	71.25	70.8
120.0	134.25	81.37	111.1
240.0	127.28	80.97	79.4
480.0	96.28	73.34	85.5
720.0	59.44	62.59	61.8

*Weight of the rat in grams.

TABLE A-10

Blood level following a multiple oral dose (40 mg t.i.d.) of ethosuximide solution administered to male Wistar rats

Time (hour)	Blood levels Rat - 32 (416 g)* mcg/ml	Rat - 33 (415 g) mcg/ml	Rat - 34 (416 g) mcg/ml
12.0	59.4	62.6	61.9
24.0	96.0	52.4	66.1
36.0	109.9	45.5	121.3
48.0	97.6	70.8	66.0
60.0	-	114.2	67.1
72.0	71.9	81.5	56.2
84.0	66.9	79.6	72.6
96.0	81.9	75.5	62.6
108.0[a]	98.7	75.9	87.5
112.0	147.9	152.9	164.2
122.0	97.0	65.9	79.5
145.0	30.6	30.5	34.8

*Weight of the rat in grams

[a]Time last dose was given.

TABLE A-11

Ethosuximide blood levels in male Wistar rats following oral administration of Formulation B, (ZARONTIN^{R} syrup 0.8 ml) equivalent to 40 mg of ethosuximide

Time (hour)	Blood levels Rat - 35 (410 g)* mcg/ml	Rat - 36 (400 g) mcg/ml	Rat - 37 (400 g) mcg/ml
0.5	35.47	94.15	42.52
1.0	54.34	100.13	60.12
1.5	62.28	107.22	82.6
2.0	70.6	115.8	106.34
3.0	74.8	117.91	96.5
6.0	76.8	100.08	87.8
9.0	68.08	85.57	76.8
12.0	56.34	81.9	68.84

*Weight of the rat in grams.

TABLE A-12

Ethosuximide blood levels in male Wistar rats following oral administration of Formulation D. (ZARONTIN^{R} Capsule contents with PEG-400) equivalent to 40 mg of ethosuximide

Time (hour)	Blood levels Rat - 38 (480 g)* mcg/ml	Rat - 39 (512 g) mcg/ml	Rat - 40 (420 g) mcg/ml
0.5	40.7	26.73	29.96
1.0	41.4	44.58	45.9
1.5	46.22	64.12	52.85
2.0	49.96	90.68	82.85
3.0	60.09	85.02	86.23
6.0	80.85	90.07	80.02
9.0	91.54	79.57	85.19
12.0	88.02	68.93	63.29

*Weight of the rat in grams.

TABLE A-13

Ethosuximide blood levels in male Wistar rats following oral administration of Formulation E, (Ethosuximide solution containing 1.5% methylcellulose) equivalent to 40 mg of ethosuximide

Time (hour)	Blood levels Rat - 41 (490 g)* mcg/ml	Rat - 42 (512 g) mcg/ml	Rat - 43 (520 g) mcg/ml
0.5	74.8	38.65	30.33
1.0	83.55	51.38	43.02
1.5	87.15	62.31	53.01
2	92.1	73.13	58.22
3	94.9	77.52	63.07
6	88.28	93.99	67.51
9	80.05	69.78	69.23
12	69.29	60.15	59.82

*Weight of the rat in grams.

TABLE A-14

Ethosuximide blood levels in male Wistar rats following oral administration of Formulation F, (Ethosuximide solution containing 10% activated charcoal) equivalent to 40 mg of ethosuximide.

Time (hour)	Blood levels Rat - 44 (420 g)* mcg/ml	Rat - 45 (460 g) mcg/ml	Rat - 46 (480 g) mcg/ml
0.5	21.29	27.04	35.21
1.0	48.08	44.18	68.33
1.5	52.55	62.15	67.06
2.0	58.09	64.8	66.33
3.0	72.15	68.05	70.69
6.0	76.58	83.70	71.04
9.0	77.5	80.64	74.77
12.0	62.4	70.04	63.95

*Weight of the rat in grams.

TABLE A-15

Ethosuximide blood levels in male Wistar rats following oral administration of Formulation G, (Ethosuximide solution in olive oil) equivalent to 40 mg of ethosuximide

Time (hour)	Blood levels Rat - 47 (417 g)* mcg/ml	Rat - 48 (460 g) mcg/ml	Rat - 49 (460 g) mcg/ml
0.5	34.13	26.47	33.08
1.0	49.7	35.21	38.23
1.5	56.18	40.21	43.26
2.0	60.07	44.53	52.16
3.0	64.52	60.31	61.25
6.0	78.28	72.52	75.03
9.0	86.19	84.23	66.1
12.0	76.88	71.11	59.67

*Weight of the rat in grams.

TABLE A-16

Ethosuximide blood levels in male Wistar rats following oral administration of Formulation H, (Ethosuximide solution containing 10% calcium triphosphate) equivalent to 40 mg of ethosuximide.

Time (hour)	Blood levels Rat - 50 (480 g)* mcg/ml	Rat - 51 (412 g) mcg/ml	Rat - 52 (380 g) mcg/ml
0.5	65.1	48.64	49.74
1.0	76.4	78.36	67.01
1.5	81.1	81.01	78.64
2.0	86.33	84.3	84.37
3.0	92.35	91.38	91.37
6.0	109.56	108.55	92.61
9.0	116.25	82.49	82.61
12.0	107.95	69.51	73.9

*Weight of the rat in grams.

TABLE A-17

Ethosuximide blood levels in male Wistar rats following oral administration of Formulation I, (Ethosuximide solution containing 10% calcium chloride) equivalent to 40 mg of ethosuximide

Time (hour)	Blood levels Rat - 53 (400 g)* mcg/ml	Rat - 54 (490 g) mcg/ml	Rat - 55 (430 g) mcg/ml
0.5	26.65	28.66	20.12
1.0	32.66	40.424	32.51
1.5	32.56	56.73	36.11
2.0	44.30	63.31	41.12
3.0	77.21	72.84	76.22
6.0	85.04	76.04	80.67
9.0	67.65	72.19	68.51
12.0	50.94	68.27	56.09

*Weight of the rat in grams.

TABLE A-18

Ethosuximide blood levels in male Wistar rats following oral administration of Formulation J, (Ethosuximide solution containing 10% calcium carbonate) equivalent to 40 mg of ethosuximide

Time (hour)	Blood levels Rat - 56 (410 g)* mcg/ml	Rat - 57 (445 g) mcg/ml	Rat - 58 (440 g) mcg/ml
0.5	58.62	34.08	50.86
1.0	62.4	50.7	61.67
1.5	61.2	76.21	62.13
2.0	63.6	75.11	70.43
3.0	65.5	67.05	73.93
6.0	60.22	73.87	71.55
9.0	53.11	70.72	74.24
12.0	47.2	33.66	65.6

*Weight of the rat in grams.

TABLE A-19

Ethosuximide blood levels in male Wistar rats following oral administration of Formulation K, (Ethosuximide solution containing 10% dried aluminum hydroxide gel) equivalent to 40 mg of ethosuximide

Time (hour)	Blood levels Rat - 59 (430 g)* mcg/ml	Rat - 60 (450 g) mcg/ml	Rat - 61 (480 g) mcg/ml
0.5	28.28	40.38	40.2
1.0	60.99	80.08	87.6
1.5	65.32	85.11	90.06
2.0	71.15	92.0	92.08
3.0	80.08	103.95	98.05
6.0	74.08	89.51	93.67
9.0	68.11	80.12	85.69
12.0	59.55	74.08	63.6

*Weight of the rat in grams.

TABLE A-20

Ethosuximide blood levels in male Wistar rats following oral administration of Formulation L, (Ethosuximide solution containing 10% magnesium trisilicate) equivalent to 40 mg of ethosuximide

Time (hour)	Blood levels Rat - 62 (450 g)* mcg/ml	Rat - 63 (420 g) mcg/ml	Rat - 64 (480 g) mcg/ml
0.5	56.43	53.88	44.51
1	77.41	62.21	58.36
1.5	80.05	74.2	69.46
2	82.63	76.35	62.23
3	97.33	80.5	73.07
6	75.72	78.81	74.89
9	61.65	74.64	69.43
12	49.82	64.08	53.87
29	25.92	31.12	28.27

*Weight of the rat in grams.

TABLE A-21

Urinary excretion data for ethosuximide in male Wistar rats

Rat No: 65 Weight (gram): 540.0 Pretreatment: Uncontrolled

Urine pH: 6.4 - 7.0 Dose: 40 mg ethosuximide solution

Time (hour)	DE_t Unchanged Drug Excreted to time T (mcg)	DE_{cum} Cumulative amount Excreted to (mcg)	%D Percent of Dose Excreted Unchanged	dDE_{cum}/dt Excretion Rate ($mcg.hr^{-1}$)
6.25	-	-	-	246.0
12.5	3082.0	3082.0	7.7	-
18.5	-	-	-	116.0
24.5	1392.0	4474.0	11.2	-
30.25	-	-	-	122.0
36.0	1430.0	5904.0	14.76	-
42.25	-	-	-	52.48
48.5	656.0	6560.0	16.4	-
54.5	-	-	-	27.16
60.5	326.0	6886.0	17.22	-
66.5	-	-	-	15.25
72.5	183.0	7069.0	17.67	-
76.75	-	-	-	10.47
81.0	89.0	7158.0	17.90	-
89.5	-	-	-	7.23
98.0	123.0	7281.0	18.3	-

TABLE A-22

Urinary excretion data for ethosuximide in male Wistar rats

Rat No: 66 Weight (gram): 560.0 Pretreatment: Uncontrolled

Urine pH: 6.4 - 7.0 Dose: 40 mg ethosuximide solution

Time (hour)	DE_t Unchanged Drug excreted to time t (mcg)	DE_{cum} Cumulative amount Excreted to (mcg)	%D Percent of Dose Excreted Unchanged	dDE_{cum}/dt Excretion Rate ($mcg.hr^{-1}$)
6.25	-	-	-	247.0
12.5	3088.7	3088.7	7.7	-
18.5	-	-	-	98.16
24.5	1178.0	4266.7	10.7	-
30.25	-	-	-	135.48
36.0	1558.0	5824.7	14.56	-
42.25	-	-	-	55.12
48.5	689.0	6513.7	16.28	-
54.5	-	-	-	60.8
60.5	729.9	7243.6	18.11	-
66.5	-	-	-	34.36
72.5	412.4	7656.0	19.14	-
76.5	-	-	-	11.84
81.0	100.7	7756.7	19.39	-
89.5	-	-	-	10.3
98.0	175.2	7931.9	19.8	-

TABLE A-23

Urinary excretion data for ethosuximide in male Wistar rats

Rat No: 67 Weight (gram): 486.0 Pretreatment: Uncontrolled

Urine pH: 6.4 - 7.0 Dose: 40 mg ethosuximide solution

Time (hour)	DE_t Unchanged Drug Excreted to time t (mcg)	DE_{cum} Cumulative amount Excreted to (mcg)	%D Percent of Dose Excreted Unchanged	dDE_{cum}/dt Excretion Rate ($mcg.hr^{-1}$)
6.00	-	-	-	198.7
12.0	2385.0	2385.0	5.96	-
18.25	-	-	-	145.3
24.5	1816.0	4201.0	10.5	-
30.25	-	-	-	56.17
36.0	639.0	4840.0	12.1	-
42.00	-	-	-	36.0
48.0	439.0	5279.0	13.2	-
54.0	-	-	-	11.8
60.0	142.0	5421.0	13.6	-
66.5	-	-	-	8.8
73.0	115.0	5536.0	13.8	-
78.70	-	-	-	3.0
84.5	34.0	5570.0	13.9	-
91.0	-	-	-	2.3
97.5	40.0	5610.0	14.0	-

TABLE A-24

Urinary excretion data for ethosuximide in male Wistar rats

Rat No: 68 Weight (gram): 457.0 Pretreatment: Uncontrolled

Urine pH: 6.4 - 7.0 Dose: 40 mg ethosuximide solution

Time (hour)	DE_t Unchanged Drug Excreted to time t (mcg)	DE_{cum} Cumulative amount Excreted to (mcg)	%D Percent of Dose Excreted Unchanged	dDE_{cum}/dt Excretion Rate ($mcg.hr^{-1}$)
6.0	-	-	-	215.0
12.0	2585.0	2585.0	6.5	-
18.25	-	-	-	102.8
24.5	1285.0	3870.0	9.7	-
30.25	-	-	-	60.6
36.0	697.0	4567.0	11.4	-
42.0	-	-	-	41.75
48.0	501.0	5068.0	12.67	-
54.0	-	-	-	19.33
60.0	232.0	5300.0	13.3	-
66.5	-	-	-	12.07
73.0	157.0	5457.0	13.64	-
78.7	-	-	-	6.08
84.5	70.0	5527.0	13.8	-
91.0	-	-	-	3.53
97.5	43.0	5570.0	13.93	-

TABLE A-25

Urinary excretion data for ethosuximide in male Wistar rats

Rat No: 69 Weight (gram): 390.0 Pretreatment: Sodium bicarbonate

Urine pH: 8.0 - 8.3 Dose: 40 mg ethosuximide solution

Time (hour)	DE_t Unchanged Drug Excreted to time t (mcg)	DE_{cum} Cumulative amount Excreted to (mcg)	%D Percent of Dose Excreted Unchanged	dDE_{cum}/dt Excretion Rate ($mcg.hr^{-1}$)
1.5	-	-	-	319.7
3.0	959.2	959.2	2.4	-
4.5	-	-	-	118.8
6.0	356.4	1315.6	3.3	-
12.5	-	-	-	89.63
19.0	1165.2	2480.8	6.2	-
23.0	-	-	-	43.78
27.0	350.3	2831.1	7.1	-
35.0	-	-	-	125.96
43.0	2015.4	4846.5	12.1	-
54.5	-	-	-	25.48
66.0	586.0	5432.5	13.6	-
72.0	-	-	-	13.85
78.0	116.3	5598.8	14.1	-
85.0	-	-	-	10.11
92.0	141.5	5740.3	14.35	-

TABLE A-26

Urinary excretion data for ethosuximide in male Wistar rats

Rat No: 70 Weight (gram): 400.0 Pretreatment: Sodium Bicarbonate

Urine pH: 8.0 - 8.3 Dose: 40 mg ethosuximide solution

Time (hour)	DE_t Unchanged Drug Excreted to time t (mcg)	DE_{cum} Cumulative amount Excreted to (mcg)	%D Percent of Dose Excreted Unchanged	dDE_{cum}/dt Excretion Rate ($mcg.hr^{-1}$)
1.5	-	-	-	299.2
3.0	897.7	897.7	2.3	-
4.5	-	-	-	160.36
6.0	481.1	1378.8	3.45	-
12.5	-	-	-	76.5
19.0	994.5	2373.3	5.93	-
23.0	-	-	-	51.8
27.0	414.3	2787.6	6.97	-
35.0	-	-	-	63.8
43.0	1021.2	3808.8	9.5	-
54.5	-	-	-	42.2
66.0	970.6	4779.4	11.9	-
72.0	-	-	-	15.5
78.0	186.2	4965.6	12.4	-
85.0	-	-	-	11.01
92.0	154.2	5119.8	12.8	-

TABLE A-27

Urinary excretion data for ethosuximide in male Wistar rats

Rat No: 71 Weight (gram): 380.0 Pretreatment: Sodium bicarbonate

Urine pH: 8.0 - 8.3 Dose: 40 mg ethosuximide solution

Time (hour)	DE_t Unchanged Drug Excreted to time t (mcg)	DE_{cum} Cumulative amount Excreted to (mcg)	%D Percent of Dose Excreted Unchanged	dDE_{cum}/dt Excretion Rate ($mcg.hr^{-1}$)
1.5	-	-	-	276.5
3.0	829.5	829.5	2.1	-
4.5	-	-	-	81.87
6.0	245.6	1075.1	2.7	-
12.5	-	-	-	71.25
19.0	926.3	2001.4	5.0	-
23.0	-	-	-	56.26
27.0	450.1	2451.5	6.13	-
35.0	-	-	-	33.36
43.0	533.7	2985.2	7.5	-
54.5	-	-	-	6.05
66.0	139.2	3124.4	7.8	-
72.0	-	-	-	11.1
78.0	132.9	3257.3	8.14	-
85.0	-	-	-	7.59
92.0	106.2	3363.5	8.4	-

TABLE A-28

Urinary excretion data for ethosuximide in male Wistar rats

Rat No: 72 Weight (gram): 400.0 Pretreatment: Ammonium Chloride

Urine pH: 5.0 - 5.5 Dose: 40 mg ethosuximide solution

Time (hour)	DE_t Unchanged Drug Excreted to time t (mcg)	DE_{cum} Cumulative amount Excreted to (mcg)	%D Percent of Dose Excreted Unchanged	dDE_{cum}/dt Excretion Rate ($mcg.hr^{-1}$)
1.5	-	-	-	82.2
3.0	246.7	246.7	0.6	-
4.5	-	-	-	160.6
6.0	481.7	728.4	1.82	-
12.5	-	-	-	136.5
19.0	1774.0	2502.4	6.25	-
23.0	-	-	-	65.15
27.0	521.2	3023.6	7.6	-
35.0	-	-	-	62.5
43.0	999.8	4023.4	10.1	-
54.5	-	-	-	25.3
66.0	582.2	4605.6	11.51	-
72.0	-	-	-	13.3
78.0	160.1	4765.7	11.9	-
85.0	-	-	-	7.2
92.0	100.5	4865.8	12.2	-

TABLE A-29

Urinary excretion data for ethosuximide in male Wistar rats

Rat No: 73 Weight (gram): 380.0 Pretreatment: Ammonium chloride

Urine pH: 5.0 - 5.5 Dose: 40 mg ethosuximide solution

Time (hour)	DE_t Unchanged Drug excreted to time t (mcg)	DE_{cum} Cumulative amount Excreted to (mcg)	%D Percent of Dose Excreted Unchanged	dDE_{cum}/dt Excretion Rate ($mcg.hr^{-1}$)
1.5	-	-	-	65.2
3.0	195.6	195.6	0.49	-
4.5	-	-	-	175.6
6.0	526.9	722.5	1.81	-
12.5	-	-	-	82.9
19.0	1077.6	1800.1	4.5	-
23.0	-	-	-	58.9
27.0	471.2	2271.3	5.7	-
35.0	-	-	-	53.9
43.0	862.4	3133.7	7.8	-
54.5	-	-	-	23.2
66.0	533.4	3667.1	9.2	-
72.0	-	-	-	11.4
78.0	137.0	3804.1	9.5	-
85.0	-	-	-	7.03
92.0	98.4	3902.5	9.7	-

TABLE A-30

Urinary excretion data for ethosuximide in male Wistar rats

Rat No: 74 Weight (gram): 390.0 Pretreatment: Ammonium Chloride

Urine pH: 5.0 - 5.5 Dose: 40 mg ethosuximide solution

Time (hour)	DE_t Unchanged Drug Excreted to time t (mcg)	DE_{cum} Cumulative amount Excreted to (mcg)	%D Percent of Dose Excreted Unchanged	dDE_{cum}/dt Excretion Rate ($mcg.hr^{-1}$)
1.5	-	-	-	65.2
3.0	195.3	195.3	0.49	-
4.5	-	-	-	157.5
6.0	472.4	667.9	2.67	-
12.5	-	-	-	110.74
19.0	1439.6	2107.5	5.27	-
23.0	-	-	-	62.2
27.0	497.4	2605.0	6.5	-
35.0	-	-	-	51.6
43.0	824.9	3429.9	8.57	-
54.5	-	-	-	24.3
66.0	559.3	3989.2	9.97	-
72.0	-	-	-	11.7
78.0	140.2	4129.4	10.3	-
85.0	-	-	-	10.51
92.0	147.1	4276.4	10.7	-

TABLE A-31

Urinary excretion data for ethosuximide in male Wistar rats

Rat No: 75 Weight (gram): 400.0 Pretreatment: Probenecid

Urine pH: 6.4 - 7.0 Dose: 40 mg ethosuximide solution

Time (hour)	DE_t Unchanged Drug Excreted to time t (mcg)	DE_{cum} Cumulative amount Excreted to (mcg)	%d Percent of Dose Excreted Unchanged	dDE_{cum}/dt Excretion Rate ($mcg.hr^{-1}$)
1.5	-	-	-	16.6
3.0	50.1	50.1	0.125	-
4.5	-	-	-	135.7
6.0	417.3	467.4	1.17	-
14.0	-	-	-	140.75
22.0	2252.1	2719.5	6.8	-
27.75	-	-	-	61.39
33.5	706.1	3425.6	8.6	-
40.5	-	-	-	28.5
47.5	399.0	3824.6	9.56	-
61.25	-	-	-	8.32
75.0	228.9	4053.5	10.1	-

TABLE A-32

Urinary excretion data for ethosuximide in male Wistar rats

Rat No: 76 Weight (gram): 444.0 Pretreatment: Probenecid

Urine pH: 6.4 - 7.0 Dose: 40 mg ethosuximide solution

Time (hour)	DE_t Unchanged Drug Excreted to time t (mcg)	DE_{cum} Cumulative amount Excreted to (mcg)	%D Percent of Dose Excreted Unchanged	dDE_{cum}/dt Excretion Rate ($mcg.hr^{-1}$)
1.5	-	-	-	392.9
3.0	1178.6	1178.68	2.95	-
4.5	-	-	-	258.6
6.0	775.9	1954.6	4.89	-
14.0	-	-	-	133.6
22.0	2137.4	4092.0	10.23	-
27.75	-	-	-	44.6
33.5	512.0	4604.0	11.5	-
40.5	-	-	-	47.2
47.5	660.4	5264.4	13.16	-
61.25	-	-	-	7.1
75.0	191.6	5456.0	13.64	-

TABLE A-33

Urinary excretion data for ethosuximide in male Wistar rats

Rat No: 77 Weight (gram): 420.0 Pretreatment: Probenecid

Urine pH: 6.4 - 7.0 Dose: 40 mg ethosuximide solution

Time (Hour)	DE_t Unchanged Drug excreted to time t (mcg)	DE_{cum} Cumulative amount Excreted to (mcg)	%D Percent of Dose Excreted Unchanged	dDE_{cum}/dt Excretion Rate ($mcg.hr^{-1}$)
1.5	-	-	-	98.2
3.0	294.5	294.5	0.74	-
4.5	-	-	-	35.5
6.0	106.6	401.1	1.0	-
14.0	-	-	-	162.4
22.0	2598.1	2999.2	7.5	-
27.75	-	-	-	54.7
33.5	629.3	3628.5	9.1	-
40.5	-	-	-	76.01
47.5	1064.2	4692.7	11.73	-
61.25	-	-	-	15.4
75.0	423.8	5116.5	12.79	-

APPENDIX B

SYMBOLS

α = Fast disposition rate constant

β = Slow disposition rate constant

A = Y intercept on senilog plot of plasma concentration versus time as shown in Figure 16, 17, 18, 19.

A_o = $\frac{FD}{V}$, the fraction of dose available at the absorption site

AUC = Area under the plasma concentration-time curve

B = y intercept on senilog plot of plasma concentration versus time as shown in Figure 16, 17, 18, 19.

$(C_o)_n$ = Concentration of drug in the blood at the start of nth dosing interval following nth dose.

C_c^o = Concentration of drug in the central compartment at t = 0 for I.V. dose.

C_c = Concentration of drug in central compartment or plasma compartment at any time t

C_{max} = Maximum plasma concentration of drug following absorption from the site of application.

D_c = Amount of drug in the central compartment.

D_t = Amount of drug in the tissue compartment.

D_o = Dose administered

D^o = Amount of drug in the central compartment at time zero or initial dose

Du = Amount of drug excreted unchanged in the urine

Dg = Amount of drug in the gastrointestinal tract

DE = Amount of drug excreted

$(DE)_\infty$ = Total amount of intact drug excreted in the urine at infinite time

D_{me} = Amount of metabolite of the drug excreted

D_{mu} = Amount of metabolite excreted in the urine

Dm = Amount of metabolite excreted in the urine

$\frac{dDE}{dt}$ = Urinary excretion rate of a drug.

$\frac{dDg}{dt}$ = Rate of change of drug in the GIT

$\frac{dDc}{dt}$ = Rate of change of drug in the central compartment.

$\frac{dDm}{dt}$ = Rate of change of amount of drug metabolite in central compartment.

F = Fraction of the dose that is absorbed and that reaches general circulation.

$\frac{FD}{V}$ = Fraction of the dose administered which is absorbed per volume of distribution

$(\frac{FD}{V})_n$ = Fraction of dose absorbed per volume of distribution from n^{th} dose

K = Overall elimination rate constant

Ka = First order absorption (availability) rate constant

Kct = First order rate constant for transfer of the drug from the central compartment to peripheral compartment

Ktc = First order rate constant for the return of the drug from the peripheral compartment to the central compartment

Ku = First order urinary excretion rate constant

Km = First order rate constant for metabolism

log = Common logarithm (base 10)

ln = Natural logarithm (base e)

t = Time after administration of dose

T = Peripheral compartment

J = Dosing interval

$t_{\frac{1}{2}}$ = Half life of drug elimination

t_{max} = Time following drug absorption when the plasma concentration reaches a maximum value

U = Urinary compartment

Vd = Apparent volume of distribution

Vc = Apparent volume of central compartment

Vt = Apparent volume of the tissue compartment

$((\frac{FD}{V})e^{-KaJ})_{n-1}$ = The fraction of dose per volume of distribution which is remaining in the gut from previous dose, for absorption

K_{TC} = Ktc (see above)

K_{CT} = Kct (see above)

C = Central compartment

K_U = Ku (see above)

D_G = Dg (see above)

K_A = Ka (see above)

APPENDIX C

A PROGRAM FOR LINEAR CORELATION AND REGRESSION

```
 E A
*C-FOCAL,69CE
*
*01.05 E
*01.09 A ! "   X",X; I (X) 1.20
*01.10 S  C=C+X;S D=D+X↑2;S N=N+1;GOTO 4.15
*01.20 S R=(H-C*E/N)/FSQT((D-C↑2/N)*(G-E↑2/N))
*01.30 S A=(H-C*E/N)/(D-C↑2/N)
*01.35 S B=(E-A*C)/N
*
*02.10 T !!"   R",%8.04,R
*02.15 T !"   D.F.",N-2
*02.20 T !!"   Y   ",A," X  +(",B,")"!!
*02.30 T !!!"DO YOU WISH TO PREDICT X FROM Y?  IF YES TYPE -1;
*02.32 T !"IF NO TYPE 0."
*02.35 A Z
*02.40 I (Z)5.10,5.20
*
*04.15 A "   Y",Y;S E=E+Y;S G =G+Y↑2;S H=H+Y*X;GOTO 1.09
*
*05.10 A !"   Y",W;I  (W)5.20;S T=(W-B)/A;T "   X",T
*05.12 GOTO 5.10
*05.20 Q
**
```

A PROGRAM FOR LOG-LINEAR REGRESSION ANALYSIS

```
E A
*C-FOCAL,69CE
*
*01.05 E
*01.09 A ! "   X",X; I (X) 1.20
*01.10 S  C=C+X;S D=D+X↑2;S N=N+1;GOTO 4.15
*01.20 S R=(H-C*E/N)/FSQT((D-C↑2/N)*(G-E↑2/N))
*01.30 S A=(H-C*E/N)/(D-C↑2/N)
*01.35 S B=(E-A*C)/N
*
*02.10 T !!"   R",%8.04,R
*02.15 T !"   D.F.",N-2
*02.20 T !!"   Y   ",A,"  X  +(",B,")"!!
*02.29 T ! "ANTILOG OF Y INTERCEPT",FEXP(B)
*02.30 T !!!"DO YOU WISH TO PREDICT X FROM Y?  IF YES TYPE -1;
*02.32 T !"IF NO TYPE 0."
*02.35 A Z
*02.40 I (Z)5.10,5.20
*
*04.15 A "   Y",Y;S Y=FLOG(Y);S E=E+Y;S G =G+Y↑2;S H=H+Y*X;GOTO 1.09
*
*05.10 A !"   Y",W;I  (W)5.20;S W=FLOG(W);S T=(W-B)/A;T "   X",T
*05.12 GOTO 5.10
*05.20 Q
**
```

```
  A PROGRAM TO CARRY OUT ANALYSIS OF VARIANCE FOR
  COMPLETELY RANDOM DESIGN HAVING UNEQUAL REPLICATION
 E A
*C-FOCAL,69CE
*
*01.01 E
*01.10 A ! "NUMBER OF TREATMENTS",T;S I=1
*01.12 T !! "ENTER DATA GROUPED BY TREATMENT"
*01.14 T ! "INDICATE END OF TREATMENT GROUP BY -1"
*01.18 A ! X;I (X)1.20;GOTO 5.01
*01.20 S I=I+1;T ! "*";I (T-I)2.01,1.18,1.18
*
*02.01 S C=SX↑2/N;S TO=CX-C
*02.03 F I=1,T; S ZX=ZX+SX(I)↑2/R(I);S DF=DF+R(I)-1
*02.05 S TR=ZX-C; S SE=TO-TR
*02.07 S A=(TR/(T-1))/(SE/(DF))
*02.10 T !!! "SOURCE          DF         SS         MS         F"
*02.12 T ! %6.03, "TREATMENTS  ",T-1,"  ",TR,"  ",TR/(T-1),"  ",A,
*02.14 T ! "ERROR        ",DF,"  ",SE,"  ",SE/(DF)
*02.16 T %6.03 !!"TOTAL        ",N-1,"  ",TO,!!
*02.17 T !"MEANS";F I=1,T;T ! I,SX(I)/R(I)
*02.18 T !"DIFFERENCE BETWEEN TREATMENTS"
*02.19 T !"GROUPS                    T          SE",!
*02.25 F I=1,T-1;F J=I+1,T;D 3
*02.26 GOTO 4.01
*
*03.03 S P=I;S Q=J
*03.05 T ! P,Q, (SX(P)/R(P)-SX(Q)/R(Q))/FSQT((SE/DF)*(1/R(P)+1/R(Q)))
*03.10 T FSQT((SE/DF)*(1/R(P)+1/R(Q)))
*
*04.01 T !!"DF",DF
*04.02 Q
*
*05.01 S SX=SX+X;S CX=CX+X↑2;S N=N+1
*05.03 S SX(I)=SX(I)+X;S R(I)=R(I)+1
*05.05 GOTO 1.18
*
*06.01 Q
**
```

```
 A PROGRAM TO CALCULATE PLASMA LEVELS FOLLOWING MULTIPLE
 DOSE REGIMEN USING ONE COMPARTMENT OPEN MODEL.
 E A
*C-FOCAL,69CE
*
*01.01 E
*01.10 A !"F",G,!"VD",VD
*01.12 A !"KE",K,!"KA",KA
*01.14 A !"TIME",T,"  DOSE",D;I (D)1.90,2.01,2.01
*01.90 Q
*
*02.01 S DI=T-TS;S TS=T
*02.10 S A0=(G*D/VD)+AS*FEXP(-KA*DS)
*02.20 S C=KA*A0*(FEXP(-K*DI)-FEXP(-KA*DI))/(KA-K)
*02.22 S C=C+CS*FEXP(-K*DI);T %8.03,"  C",C
*02.24 I (D)1.90,2.30,2.40
*02.30 S CS=C;S DS=DI;S AS=A0;GOTO 1.14
*02.40 S M=(KA↑2*A0)/(K*(KA*A0+(KA-K)*C))
*02.42 S TM=FLOG(M)/(KA-K)
*02.44 S CM=(A0*KA/K)/FEXP((KA/(KA-K))*FLOG(M))
*02.46 T "  TM",TM+T,"  CM",CM;GOTO 2.30
**
```

```
 A PROGRAM TO CALCULATE PLASMA LEVELS FOLLOWING MULTIPLE DOSE REGIMEN
 IN TWO COMPARTMENT OPEN MODEL
 E A
*C-FOCAL,69CE
*
*01.01 E
*01.10 A !"F",G,!"V1",V1,!"V2",V2
*01.15 A !"KA",KA,!"KBT",KB,!"KTB",KT,!"KF",K
*01.17 S A=0.5*((KB+K+KT)+FSQT((KB+K+KT)↑2-4*KT*K))
*01.18 S B=0.5*((KB+K+KT)-FSQT((KB+K+KT)↑2-4*KT*K))
*01.20 A !"TIME",T,"  DOSE",D;I (D)1.29,2.01,2.01
*01.29 Q
*
*02.01 S DI=T-TS;S TS=T
*02.10 S A0=G*D+AS*FEXP(-KA*DI)
*02.12 S P1=M1;S Q1=M2
*02.15 S P2=M1*(KA+KT)+KT*M2+KA*AS
*02.20 S P3=KA*KT*(AS+M1+M2)
*02.25 S Q2=M2*(KB+K+KA)+KB*M1
*02.30 S Q3=KA*KB*(AS+M1+M2)+KA*K*M2
*02.35 S M1=((P2*A-P1*A↑2-P3)/((A-B)*(KA-A)))*FEXP(-A*DI)
*02.37 S M1=M1+((P2*B-P1*B↑2-P3)/((A-B)*(B-KA)))*FEXP(-B*DI)
*02.39 S M1=M1-((P1*KA↑2-P2*KA+P3)/((B-KA)*(KA-A)))*FEXP(-KA*DI)
*02.40 S M2=((Q2*A-Q1*A↑2-Q3)/((A-B)*(KA-A)))*FEXP(-A*DI)
*02.42 S M2=M2+((Q2*B-Q1*B↑2-Q3)/((A-B)*(B-KA)))*FEXP(-B*DI)
*02.44 S M2=M2-((Q1*KA↑2-Q2*KA+Q3)/((B-KA)*(KA-A)))*FEXP(-KA*DI)
*02.50 S AS=A0
*02.55 T "  C1",M1/V1,"  C2",M2/V2;GOTO 1.20
**
```

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(1) Masih, S.Z., and Block, L.H., Influence of Internal Hydrostatic Pressure on In Vitro Release of Triamcinolone Acetonide from Carbopol Gel Base.

(2) Masih, S.Z., and Biggs, D.F., A Simple, Accurate and Reproducible GLC Method for Analysis of Ethosuximide, Methsuximide, and Phensuximide in Plasma, Urine and Tissue Homogenates.

(3) Masih, S.Z., and Biggs, D.F., Pharmacokinetics of Ethosuximide, Methsuximide and Phensuximide following I.V. Administration in Wistar Rats.*

(4) Masih, S.Z. and Biggs, D.F., Pharmacokinetics of Ethosuximide Following Single Oral and Multiple Oral Doses in Wistar Rats.

(5) Masih, S.Z., and Biggs, D.F., Urinary Excretion Kinetics of Ethosuximide in Wistar Rats.**

(6) Masih, S.Z., and Biggs, D.F., Influence of Some Pharmaceutical Adjuvants and Antacids on Systemic Availability of Ethosuximide in Wistar Rats.

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**Presented in part at the 121st Annual Meeting, American Pharmaceutical Association, August 3 - 8, 1974, Chicago, Illinois.

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